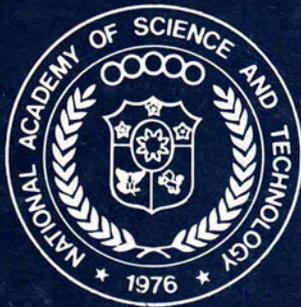


**PROCEEDINGS**  
of the  
**INTERNATIONAL CONFERENCE**  
**ON FOOD PRESERVATION AND**  
**SECURITY**  
**Republic of the Philippines**

**November 8-11, 1994**

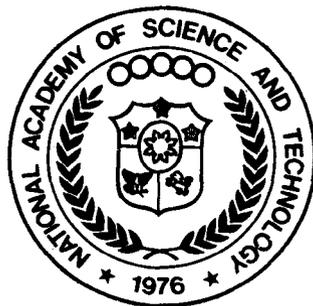


Republic of the Philippines  
National Academy of Science and Technology  
TAPI Building, DOST Complex, Bicutan,  
Taguig, M.M.



**PROCEEDINGS**  
**of the**  
**INTERNATIONAL CONFERENCE**  
**ON FOOD PRESERVATION AND**  
**SECURITY**  
**Republic of the Philippines**

**November 8-11, 1994**



**Republic of the Philippines**  
**National Academy of Science and Technology**  
**TAPI Building, DOST Complex, Bicutan,**  
**Taguig, M.M.**

National Academy of Science and Technology  
Bicutan, Taguig, Metro Manila  
Philippines

Copyright 1994 by  
The National Academy of Science and Technology

*All Rights Reserved*

No part of this book may be reproduced without written permission from the Academy, except for brief excerpts or quotations in connection with the text of a written paper or book.

**ISBN 971-8538-29-1**

Printed in  
THE REPUBLIC OF THE PHILIPPINES

# Contents

## FOREWORD

## OPENING CEREMONIES      November 8, 1994

### WELCOME ADDRESS

*William G. Padolina, Ph.D., Secretary,  
Secretary, Department of Science and Technology* ..... 3

### KEYNOTE ADDRESS

*Edgardo J. Angara, Senate President* ..... 5

## FIRST PLENARY SESSION

### AGRICULTURAL DEVELOPMENT AND FOOD SECURITY

*Amir Muhammed, Ph.D.  
President, Pakistan Academy of Sciences Islamabad, Pakistan* ..... 11

## SESSION 1: FOOD QUALITY, SAFETY AND SECURITY

### MICROBIOLOGICAL CONTROL OF AFLATOXIGENIC FUNGI IN FOODS: AN EXPLORATORY STUDY

*R.C. Mabesa, M.J.V. Sumague, R.D. Divina,  
R.D. Lauzon, V. Sabariah, P.P. de Guzman, A. Villena  
and R.R. Melendes* ..... 29

### OCCURRENCE OF FOOD-BORNE PATHOGENS IN LAGUNA KESONG PUTI AND PASTILLAS DE LECHE

*V. L. Barraquio, P. A. Pacanot, Ma. C. dP. Trinos,  
and E. N. Almazan* ..... 43

### FRESHNESS ASSESSMENT OF FISH MEAT USING THE TORRYMETER, IMPEDANCE ANALYZER AND K VALUE

*M. Sakaguchi, A. Koike, S. Takahashi and K. Kato* ..... 49

### PUBLIC HEALTH ASPECTS OF FOOD IRRADIATION

*Gerald Moy, Ph.D. and F. K. Kaferstein* ..... 55

### DEVELOPMENT OF RAPID DETECTION METHODS FOR FOOD BORNE PATHOGENS IN HACCP PROGRAMS

*M.M. Garcia, B.W. Brooks, K.H. Nielsen, A.S. Denes and E.M.  
Pietrzak* ..... 69

ANALYTICAL TOOLS FOR THE EVALUATION OF FOOD QUALITY AND SAFETY <i>Agnes C. Torres</i> .....	93
FOOD SAFETY - A VITAL ELEMENT OF FOOD SECURITY <i>Quintin L. Kintanar, M.D., Ph.D. and Carmina J. Parce,</i> .....	105
NUTRITION CONSIDERATIONS IN FOOD SECURITY: THE CASE OF THE PHILIPPINES <i>Rodolfo F. Florentino, M.D., Ph.D.</i> .....	113

**SESSION 2: FOOD PROCESSING, PACKAGING EQUIPMENT  
AND MACHINERY**

SIMULTANEOUS BIOCONVERSION OF CARBOHYDRATE AND BIODEGRADATION OF CYANIDE IN CASSAVA MEAT TO INCREASE NUTRITIVE VALUE <i>Chay B. Pham, Laura J. Pham and Teresita J. Ramirez</i> .....	139
PRODUCTION OF DIETARY FIBER FROM BAGASSE <i>Rosita P. Ebron, Rosemarie S. Gumera, Rosalina B. Tan &amp; Anglina, M. Lojo</i> .....	155
PASSIVE SOLAR ENERGY SYSTEM FOR CROP AND FOOD DRYING <i>Samuel S. Franco, Reynaldo C. Castro, Carlo S. Sambo, Heraldo L. Layaoen, Percival O. Libed, Jr., Jerry C. Ragudo and Benjamin S. Mercado, Jr.</i> .....	167
FRUIT AND VEGETABLE PROCESSING TECHNOLOGIES FOR VILLAGE-TYPE PROCESSING PLANTS <i>Nerius I. Roperos, Virginia TD. Pacaba and Lydia M. Zara</i> .....	179
DRYING CHARACTERISTICS OF SWEET POTATO SLICES <i>Lemuel M. Diamante</i> .....	187
ESTABLISHMENT OF COMMUNAL FOOD PROCESSING CENTERS IN THE REGIONS <i>L.M. Marero, A.M. Martin Jr. and M.P.E. De Guzman</i> .....	201
EVAPORATIVE COOLING STORAGE OF TOMATO AND SWEET PEPPER FRUITS HARVESTED AT DIFFERENT STAGES OF RIPENESS <i>A. L. Acedo Jr., F. G. Enriquez and M. A. Mante</i> .....	213

DEVELOPING SUGARCANE-FRUIT JUICE MIXES <i>Rosemarie S. Gumerá, Rosita P. Ebron and Marcelino M. Guevarra</i> .....	229
---------------------------------------------------------------------------------------------------------------------------	-----

**SECOND PLENARY SESSION      November 9, 1994**

FOOD IRRADIATION: INTERNATIONAL TRADE OPPORTUNITIES <i>George G. Giddings and Paisan Loaharanu</i> .....	245
----------------------------------------------------------------------------------------------------------------	-----

**SESSION 3: FOOD PRESERVATION TECHNOLOGIES**

PROSPECTS OF FOOD IRRADIATION IN THE HANDLING OF HORTICULTURAL PERISHABLES <i>Ma. Concepcion C. Lizada Ph.D.</i> .....	255
------------------------------------------------------------------------------------------------------------------------------	-----

GAMMA IRRADIATION OF SELECTED SPICES <i>Zenaida M. De Guzman, Jean M. Casyao, Mitos M. Tolentino and Luzviminda M. Ignacio</i> .....	261
---------------------------------------------------------------------------------------------------------------------------------------------	-----

FOOD IRRADIATION IN A DEVELOPING COUNTRY <i>Alicia O. Lustre, Ph.D.</i> .....	275
----------------------------------------------------------------------------------	-----

LUMINOUS BACTERIAL PRODUCT: A POTENTIAL INDUSTRIAL FOOD DYE <i>Prima C. Ragudo-Franco and Kenneth H. Neelson</i> .....	285
------------------------------------------------------------------------------------------------------------------------------	-----

DEVELOPMENT OF A NEW PROCESS FOR COCONUT OIL EXTRACTION <i>Chay B. Pham, Merlita T. Punzal, Arlene R. Moreno, Lilia M. Soriano and William G. Padolina</i> .....	299
----------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----

THE DEVELOPMENT AND OPTIMIZATION OF A COCO-SWEET POTATO CANDY <i>L.S. Palomar, D.D. Atok and M.E.M. Abit</i> .....	315
--------------------------------------------------------------------------------------------------------------------------	-----

FLAVOR ENCAPSULATION IN CARBOHYDRATE MATRICES <i>A. Blake, P. Attwool and J. Grange, M. Lindstrom,</i> .....	327
-----------------------------------------------------------------------------------------------------------------	-----

COMPARATIVE PROFITABILITY OF TWO METHODS OF CUTTING CARCASES OF CATTLE AND SWINE <i>Lourdes S. Rivera, Amorfiná G. Galaraga</i> .....	339
---------------------------------------------------------------------------------------------------------------------------------------------	-----

POST-HARVEST HANDLING AND PACKAGING OF  
THE THREE VARIETIES OF ANNATTO

*Adoracion A. Ceniza and Virginia TD. Pacaba* ..... 349

SUGAR QUALITY SPECIFICATIONS, SUGAR HANDLING,  
STORAGE AND DISTRIBUTION

*Agnes G. Collado and Nora I. Chinjen* ..... 375

**THIRD PLENARY SESSION      November 10, 1994**

THE APPLICATION OF BIOTECHNOLOGY IN FOOD SECURITY

*Gregory C. Gibbons and Carl J. Gibbons* ..... 391

**SESSION 4: FOOD INGREDIENTS AND ADDITIVES**

PRODUCTION OF LACTIC ACID BACTERIA FOR FOOD  
INOCULANT AND PRESERVATION

*Laura J. Pham, Chay B. Pham and Dominic D. Villa* ..... 403

EASTMAN SAIB-SG FOR BEVERAGE APPLICATIONS

*Lee Lai See* ..... 413

FOOD HANDLING, STORAGE AND DISTRIBUTION

*S.C. Andales, E.S. Manebog and M.C. Bulaong* ..... 419

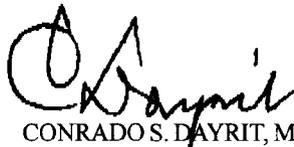
## FOREWORD

The National Academy of Science and Technology (NAST), with the assistance of the Department of Science and Technology (DOST), Food and Nutrition Research Institute (FNRI), the Philippine Nuclear Research Institute (PNRI), the Philippine Council for Industry and Energy Research and Development (PCIERD), the Philippine Association of Food Technologists, Inc. (PAFT), and the NAST Foundation, Inc., organized and coordinated the International Conference on Food Preservation and Security in response to food security problems posed by the increase of Philippine population.

The International Conference was held in Mandaluyong City, Philippines on November 8 to 11, 1994, co-sponsored by the Federation of Asian Scientific Academies and Societies (FASAS) and the Third World Academy of Sciences (TWAS). Several foreign experts presented papers on food preservation and emerging food technologies. About 350 participants attended the conference.

This proceedings, a by-product of the conference, contains 3 plenary papers and 24 papers. It highlights the importance and the new technologies on food production and preservation throughout the world.

It is hoped that this Proceedings will be useful to government officials, who make policies and allocate funds, as well as to producers and processors of food.



CONRADO S. DAYRIT, M.D.

President

National Academy of Science and Technology

# **Opening Ceremonies**

**November 8, 1994**

## WELCOME ADDRESS

*William G. Padolina, Ph.D.  
Secretary, Department of Science and Technology*

Honorable Angara, Dr. Mabesa, Dr. Dayrit and Dr. Acevedo, a pleasant good morning to you and to our distinguished colleagues in S and T. A warm welcome to one and all especially to our foreign luminaries to this 1st International Conference on Food Preservation and Security. Your presence is indeed a boost to our regional efforts at enhancing food security.

I commend the National Academy of Science and Technology for taking this step towards addressing food sufficiency issues in Asia through the promotion of appropriate food technologies and gearing individual and institutional efforts towards technology research and application. I believe that our effective networking with the Federation of Asian Scientific Academies and Societies (FASAS) and with the Third World Academy of Sciences (TWAS) has resulted in the organization and implementation of this conference. I note the interesting line-up of scientists for our plenary sessions on food security, food irradiation and food biotechnology interspersed in-between technical sessions on food quality and safety, food processing, food handling, storage and distribution, food preservation, food ingredients and additives, and packaging.

STAND capitalize on the export market, basic domestic needs, our support industries and our coconut industry.

Food and nutrition research, which is one of the DOST's sectoral concerns has, for sometime now, harped on food technology as an indispensable key to improving the health and nutrition of our people towards improved quality of life and reducing the menace of poverty. This phenomenon is felt in most developing countries as well as among the highly progressive ones represented by our international scientists here.

One salient feature in fact, of the country's Philippine Plan of Action in Nutrition which is likewise aligned to global development imperatives points to the promotion of household food security as paramount in our national development concerns. Food security is likewise echoed prominently in the World Declaration on Nutrition where distinguished world leaders assessed and set policies on improving the nutrition situation in all countries. This forum is apt for such and you are all badly needed now.

Our "exportable" winners in STAND include marine products, specifically shrimps, prawns, tuna, crabs, carageenan and seaweeds. Through this, we can conquer the world market. Fruits, whether fresh, dried or processed in this tropical paradise spell dollars (\$) and these include our very tasty bananas, mangoes and pineapples, dried papayas and our exotic durian. These are "premium" products in advanced countries and are scarce so we should go into the technology

of these products. This, I believe, is one area where we can creatively and scientifically make a difference, and establish a “niche” in search of global recognition. Will your inputs in this important scientific forum STAND to capture profitable markets?

Even our basic domestic needs are mounting and you can very well provide this generation and the next with better food under better environmental conditions for again, longer life and security. Specifically, this forum can help each of us identify and research on sure winners in the global market. How do we go about searching for appropriate technologies for the domestic and world markets? How do we share resources, expertise and equipment to make food technology at par if not better with our developed counterparts? There will be nothing more updated and more recent than the advances you will share fruitfully during this conference. I hope your collaborative arrangements will work for strengthened networking on technology R and D in the region. This conference, I hope will not only display individual scientific expertise but will trigger strategic planning towards more focused and feasibly implementable R and D that can be substantially felt in a highly competitive and critical environment. Looking at all of you now listening to me is giving me hope. I know we are still one in our goal and vision towards a better world, a better Asia, and a better Philippines for our colleagues here. Our S and T direction has never been so challenging as it is now and your individual and committed concern towards assuring food security will surely make us STAND to that challenge which we hope will bear good fruit before the year 2000. Again, to one and all, welcome and good day!

---

1 Presented at the “International Conference on Food Preservation and Security”, Manila, Philippines on November 8-11, 1994

2 Secretary, Department of Science and Technology, Bicutan, Taguig, Metro Manila, Philippines

## KEYNOTE ADDRESS

*Senate President Edgardo J. Angara*

My message here today is plain and simple:

In our time, self-sustaining growth depends on technological modernization. And events and trends in the region and in the world all compel us to modernize our technology.

Our economy shall not survive the regime of free trade being forced on us by the Uruguay Round Agreements and our membership in AFTA and APEC — unless we begin — now — to modernize our industrial, agricultural and service technologies.

In fact, the entire concept of development pioneered by our neighbors in East Asia — which has been aptly called industrialization by learning — is founded on technological modernization.

If we are to begin to catch up with our neighbors and partners in the world's fastest-growing region, we need to do two things:

One — Improve our scientific and technological education by investing more public money and private interest in it.

Two — Raise the incentives and rewards for our scientists and technologists.

To these two tasks this Academy must focus its efforts, exercise its influence — and take up its leadership role.

Let me elaborate on this theme.

### LAGGING INDUSTRIAL PRODUCTIVITY

Of course you don't need me to tell you how badly we have lagged behind our neighbors in productivity these past 40 years. The Chinese extract five times more income from a hectare of land than our own farmers do. The South Koreans, Taiwanese, and Japanese do even better.

As for Philippine manufacturing, it is "notorious for its low productivity", notes the economist Gonzalo Jurado.

There are apparently two basic reasons for this. One is that our capital imports have tended to consist of outmoded or frankly secondhand machinery — instead of cutting-edge technology. The other is that even if it were, our indus-

tries did not have the complementary software and human skills needed to exploit the new technology to the limit.

### **EVENTS AND TRENDS NOW COMPEL US TO MODERNIZE OUR TECHNOLOGY**

The reason our industrialists were satisfied with second or third-best machinery was simple. During the long period of import-substituting-industrialization, their highly protected industries were under no pressure to modernize, since they had a lock on the home market.

But, even for monopolists, all good things must come to an end. Now that we're committed to opening up the economy, our national industries have no alternative but to modernize their technology. They must begin to do as the East Asian tigers did.

How did the tiger-economies of Japan, South Korea, Taiwan, Singapore, Hongkong, Malaysia — and now Thailand, Indonesia and coastal China — get where they are?

They got to the eminence they occupy by industrialization through learning.

As we know, the process of industrialization began in Great Britain toward the end of the 1700s. Before that time, the world subsisted largely on farming and on the trade of handmade goods.

The second industrial revolution — in Germany and the United States — took place some 100 years later, toward the end of the 1800s.

Both industrial revolutions — the first and the second — produced new things and new ways of making them.

The British invented new products and new processes.

The Germans and the Americans introduced changes and new ideas based on what the British had invented.

### **INDUSTRIALIZATION BY LEARNING**

By contrast, late industrialization — such as the tiger economies accomplished, beginning in the 1950s — required no invention and no innovation. The East Asian countries grew simply by applying skillfully the processes already known in the West.

In a word, they industrialized by learning. Development in East Asia was organized by bright people — bright people with sound technical and scientific learning — who studied thoroughly the processes the West discovered — and adopted them for their own purposes.

This is why, all over East Asia — except perhaps in our own country — national policy values technical competence in engineering and science well above the liberal arts and the training of lawyers.

As we know, Japan's population is only half as large as that of the United States. But Japan graduates as many engineers. In 1986, it had 80,000 engineering graduates employed in industry; Hitachi alone had 600 doctors in engineering.

If we are to industrialize, if we are to develop, we must begin to do the same — to nurture and to reward bright young people with sound technical and scientific learning.

### **EDUCATED PEOPLE THE NEW WEALTH OF NATIONS**

Everywhere in the world, scientifically-educated people have become the new wealth of nations. The time has come when technologically-trained and technologically-educated people must also become our best national resource.

The wealth locked up in our soil, our mountains, and our surrounding seas does help. But natural resources are no longer the key elements in a nation's greatness.

If they were, the Arabs of the Middle East would be the dominant peoples of this planet; and the Japanese, Koreans, and Taiwanese among the poorest.

People with technological skills and education must become our country's greatest resource — as they are in the most progressive countries of the globe.

To gear education more closely to economic development, scientific and technological learning must become the decisive element in our education system.

### **MODERNIZATION OF TECHNOLOGY FOR FOOD SECURITY**

In the particular case of agriculture, scientists and technologists are rightly guided by the goal of achieving food security not only at the household or village level, but also for the whole nation.

Food security lies to a very large extent not only on the production of agricultural crops, but also on improved food processing technologies. What developments have we made in post-harvest technologies such as drying grain, or treating fruits and vegetables, and even spices? How can we ensure that the food we eat is safe, free from harmful microorganisms, and of high quality in terms of flavor, color, and texture? How can we extend the shelf lives of food so that they can reach the consumers before spoilage? How can the nutritional value of food items be enhanced in order to solve our nagging problems of micronutrient deficiencies and energy malnutrition?



**First Plenary Session**  
**FOOD SECURITY**



# AGRICULTURAL DEVELOPMENT AND FOOD SECURITY

*Amir Muhammed, Ph.D.  
President, Pakistan Academy of Sciences  
Islamabad, Pakistan*

## ABSTRACT

Food is essential to sustain life and developing countries (DCs) generally give high priority to achieve food security for their populations. Food availability, distribution and consumption are important parameters for achieving food security. Self-sufficiency in the main staple cereal (viz. rice, wheat or maize) through indigenous production is a desirable goal to reduce huge import costs.

The population of the DCs is increasing rapidly; of the 2.8 billion increase in global population from 1990 to 2020, nearly 94% (2.64 bn) will be in the DCs. This increased population will exert an unbearable pressure to produce enough food for their burgeoning population, especially when the land and water resources for agriculture are also shrinking due to increasing non-agricultural uses. This is the dilemma of less resources for more people and more production from less resources.

The silver lining to this gloomy scenario is that the potential of the available land and water resources for agricultural production is much higher (nearly 3-fold) compared to the present levels. Transforming agricultural production to science-based industry would result in rapid yield increases of the main crops. A nearly 4-fold increase in the average national yield of wheat in the UK from 1850 to 1990, and the large increases in rice, wheat and maize yields in several countries, especially in Asia in the wake of the Green Revolution are good examples of transformation in agriculture through utilization of technologies developed as a result of systematic scientific research.

The International Agricultural Research Centers (IARCs) established by CGIAR have been instrumental in developing improved technologies, in partnership with strengthened national agricultural research systems, which have resulted in large yield increases of major cereal crops in many DCs. It is emphasized that the main option for the DCs to achieve a healthy measure of food security during the next 2-3 decades is through increased investment in the agriculture knowledge system consisting of education, research and extension to develop improved, cost effective, location-specific technologies for a sustainable increase in productivity from the limited land and water resources.

Increased production *per se* will not result in increased food availability unless the staggering harvesting, storage and processing losses are also reduced. Therefore along with emphasis on increasing agricultural production through use of improved production technology and management, the R&D efforts on reducing losses during harvesting and storage of crops and processing of food for human consumption have also to be emphasized. The IARCs have generally neglected the post-harvest aspects which need to be included in their research agenda to achieve the overall goal of reducing malnutrition and hunger in the DCs. This, of course, would require increased financial support from the donors for the IARCs which unfortunately has been declining during the last few years.

## INTRODUCTION

Food is a basic human necessity. Adequate and balanced food intake is a prerequisite to achieve proper nutritional status to promote a healthy, creative and vigorous life for societal welfare. No wonder therefore that governments especially in the food-deficit countries lay a strong emphasis on providing sufficient food to their population. As a minimum, the top priority of the governments is ensure sufficient stocks and an adequate distribution system for the main staples. Food policies are also developed to ensure that the low income strata have access to adequate quantities of the main cereal (wheat, rice or maize, *etc.*) at reasonable prices that are often subsidized. Governments in Third World countries, whether elected or imposed, are sensitive to the extremely unpleasant consequences of food shortage which often lead to rioting, general breakdown of law and order and unceremonious change of governments. Given the overwhelming political and socioeconomic importance of the subject, governments give high priority to develop food and agriculture policies tailored to their peculiar situation, that would ensure a healthy measure of food security. The present seminar which deals with an important aspect of food security (food preservation and reduction of post-harvest losses) is therefore of great importance and interest to the developing countries (DCs).

### Food Security Perspective

The perspective of food security at family, community, national, regional and global level is often quite different. The term food security has been defined in several ways depending on the focus on selected parameters and target groups. The usually accepted definition of national food security is access by all people, at all times to enough food for an active, healthy life .

The essential elements of this definition are availability of food and ability to acquire it. Another definition relevant to developing countries is ability of food-deficit countries, or regions, or households, within those countries to meet target levels of consumption on a yearly basis<sup>2</sup>. The main issues in this definition are the identification target group and the determination of adequate consumption levels for different groups.

### Food Security Parameters

The important parameters to achieve food security are supply, distribution and consumption. Food distribution and consumption are vital to ensure food security especially for the underprivileged low income groups and involve elaborate arrangements for development of infrastructure for marketing and transportation. However, the foundation for a sound food security system lies in arranging adequate supplies to meet the overall national requirement through indigenous production and trade. Since DCs often face severe resource constraints to

purchase large quantities of food from international markets, the really effective option to achieve adequate food security for them is through indigenous production. It must however be mentioned that the New Economic Order and GATT emphasize promotion of trade based on comparative advantage for overall economic development and food security. Developing countries should be sensitive to these international trade concerns and should formulate policies which ensure an acceptable degree of food security mainly through increased productivity of the principal food crops well adapted to their agro-ecologic conditions.

This paper examines the food security situation of DCs, the perspective of agricultural development during the last few decades, and future options to increase productivity especially of food crops through sustainable development.

### **Population Growth and Food Requirement**

Most developing countries are already food-deficient and are forced to import increasingly larger quantities of food to meet the needs of the rapidly increasing population. The world population is projected to increase from 5.3 billion in 1990 to 6.3 billion in 2000 and 8.1 billion in 2020<sup>3</sup>. Of the 2.8 billion increase in global population from 1990-2020, nearly 94 percent (2.64 billion) will be in DCs whose population is projected to increase from 4.1 to 6.8 billion during this period. This increased population will put tremendous pressure on the limited land and water resources of these countries to produce more food. In addition to the urgent need to produce more food to meet the basic nutritional requirement of the additional population, efforts will have to be made to improve the nutritional status of the large numbers of the undernourished in the present population. Increasing urbanization and higher family incomes as a result of economic development necessitate production of higher quality foods for the population. All these factors put together would mean that the overall agricultural production in the developing countries will have to be increased by 100-150 percent level to meet the food needs of the population during the next quarter of a century<sup>4</sup>. This is an inescapable reality and presents a formidable, if not an impossible challenge.

### **Land and Water-the Shrinking Resource Base**

The primary sources of agricultural production are land and water. Most of the food quality land and easily harnessable water resources are already being used for agricultural production. Increasing population and urbanization would require additional land (usually high quality agricultural land in the vicinity of existing settlements) and diversion of water resources from agriculture. FAO estimated that non-agricultural uses of land for housing, transport, industry, mining and recreation *etc.* consumed 98 million hectares or 1.5 percent of the total land area of the world in 1975, which is projected to increase to 180 million hectares (2.8 percent of total area) by 2000<sup>5</sup>. Non-agricultural uses of land in the densely populated SE Asian region are estimated to consume 11 percent of the

land area. Current availability of cropland area per person estimated at 0.28 ha (0.69 acres) is expected to decline to 0.17 ha (0.42 acres) by the year 2025 on the current projections of population increase and cropland use. We thus have the dilemma of producing substantially more agricultural products from a reduced resource base *i.e.* less resources for more people and more production from less resources<sup>6</sup>.

There has been increasing concern during the last decade that efforts at economic development, especially to increase agricultural production in DCs result in pollution of the environment and degradation of the resource base resulting in an overall unsustainable agricultural and economic development. Increase in agricultural production has come about mainly through intensive cropping with increasing use of chemical inputs and irrigation and expansion on marginal areas. Increasing deforestation, desertification, salinity and waterlogging and soil erosion are the manifestations of these efforts to increase agricultural production to meet the requirements of the burgeoning population<sup>7</sup>. Agenda 21 has emphasized urgent action at national and global level to promote measures for sustainable development and check the essentially irreversible damage to the land and water resources, and the environment<sup>8</sup>.

Rural poverty is an important factor leading to unsustainable resource use. To eke out a living from their extremely limited land and water resources, the rural poor resort to measures which result in temporary increase in family income, often at the cost of damage to the resource base and pollution of the environment. They often have few alternatives to increase their family income and have to resort to unsustainable resource use, even if they are conscious of the harmful effects on future productivity. In many cases, the choice is between sustainable development yielding low incomes that further aggravate rural poverty and compromising the prospects for the future generations by unsustainable use of the available resources resulting in their degradation to increase the family income for short periods. There are no easy answers to these challenges.

### **Prospects for Increasing Agricultural Production**

The practical option to achieve food security in DCs with a large percentage of rural population and a predominantly agrarian economy is through a sustained increase in overall production of the principal cereal crops. While milk, meat and fish are important in human nutrition, the cereals are the main source of calories, and in many cases protein as well, for the large rural poor population. Therefore prospects for increasing production of the principal cereals *viz.* wheat, rice and maize are discussed in some detail. Food legumes, oilseeds, tubers, fruits and vegetables, although important for the overall agricultural development, do not play a decisive role in determining national food security and are therefore not dealt with in this article.

Cereals occupy the largest cultivated area in most DCs and constitute the dominant commodity in the overall agricultural economy. Increased production can be achieved either through expansion in the cultivated area or through agri-

cultural intensification resulting in increased production per unit area. The former option is not available to most developing countries, at least in Asia and Africa's since the available resources of good quality land and water are already shrinking. Therefore major emphasis to increase agricultural production in most DCs, at least in the short to medium term, is through agricultural intensification with emphasis on increasing the average yield of major crops on better quality land. This can only be done through judicious application of improved production technology developed through well organized research<sup>9</sup>. The culture of agriculture has to be transformed from tradition-based to science-based to achieve this breakthrough in productivity. The Green Revolution of the mid-sixties amply demonstrated that this is indeed possible.

Even before the green revolution, the industrially developed countries of today had experienced agriculture revolution in the early part of this century which was essentially based on application of scientific discoveries in the fields of agricultural chemistry and plant genetics that resulted in development of chemical fertilizers and high yielding varieties through cross-breeding. Thus the national average yield of wheat in United Kingdom registered a more than 4-fold increase from 1680 kg/ha in 1850 to 6955 kg/ha in 1990<sup>10</sup>. Average yield of rice in Japan increased more than 3 fold from less than 2 ton/ha to more than 6 ton/ha during the same period. There was also a similar increase in average maize yield in several European countries, notably Italy while in the US the maize yield increased phenomenally after the release of hybrid maize varieties since 1930.

The scientific discoveries which gave rise to large yield increases and a prosperous agriculture in Europe, North America and Japan during 1850-1950 were not utilized in the rest of the world where crop yields remained stagnant or declined. Most of the food requirements of these countries were met by bringing additional areas under cultivation. This equilibrium was disturbed when population growth started outstripping the rate of increase of food production thereby resulting in food shortages and famine like conditions in several countries. These countries had very modest capabilities for agricultural research and thus could not utilize the discoveries made in other parts of the world to achieve similar increase in crop yields. After the Second World War most of these DCs which used to be food surplus and earned their foreign exchange from food exports had to resort to imports. The situation continued to aggravate in the fifties with rapid increase in population of these countries and increasing food imports resulting in huge national debts and aggravating economic situation.

### **International Agricultural Research**

In this background of worsening food situation in the developing countries and their weak capability to generate improved technology to improve agricultural production, the World Bank, UNDP and FAO co-sponsored the creation of a Consultative Group for International Agricultural Research (CGIAR) in 1961 initially to undertake research on wheat, rice, maize and beans for the developing

countries. The impact of this research on the yield of these crops was dramatic. Encouraged by the success in increasing food production in many developing countries especially in Asia, the CGIAR attracted more donors and the number of International Agricultural Research Centers established by the CGIAR increased to 18 in 1993 since the establishment of International Rice Research Institute in 1960 (Table 1).

Besides directly transferring improved technology to the DCs through adaptive research, the CGIAR in collaboration with bilateral and multilateral donors also helped in developing national capacity in agricultural education, research and extension.

### **Yield Takeoff in Developing Countries**

Many DCs were able to achieve a "takeoff" in yield increases of major food crops as a result of these developments. Plucknett<sup>10</sup> has collected the yield data of several regions and countries on major cereal crops since 1961 which shows unprecedented continuous increase in yield since the introduction of technologies developed initially by the CGIAR centers. Thus the annual yield increase in rice after the take-off year for Asia, Oceania, Latin America and Africa has been 65, 88, 40 and 40 kg/ha (Table 2) while the corresponding yield increase for several countries has been as high as 195 (Korea DPR) and 410 kg/ha (Chad).

In case of wheat, the highest average national yield in 1990 was that of Ireland (8200 kg/ha) which also had the highest yield gain of 285 kg/ha since takeoff in 1976 (Table 3). Among the developing countries, Egypt and Chile had the highest annual yield gain since takeoff (128 and 175 kg/ha respectively).

The highest average national yield of maize in 1990 was in Chile (8200 kg/ha) which also had one of the highest annual yield gain after take-off (300 kg/ha) Table 4. Sorghum is the only major cereal crop where there has been a yield loss of about 10 kg/ha on a global basis during the decade of 1980-1990 in spite of advancements in production technology and demonstration of increased yield potential at experiment stations. Part of the reason is that sorghum has been pushed to harsher environments with increase in maize acreage that has resulted in a decline in sorghum yield in spite of availability of improved technology.

### **Yield Gap**

The objective of science-based agriculture is to bridge the large gap between potential yield obtained through optimum utilization of the available land and water resources and the average yield obtained in the farmers fields. Different levels of yield have been identified for various crops (Figure 1). The lowest is the yield obtained by average farmers who use conventional production technology and face many constraints which have been well identified by systematic research. The next category is the yield obtained by "progressive" farmers who use the recommended package of technology and apply the necessary inputs at

the proper time. The difference between these two yields is yield gap 1. Next level is the yield that is obtained at the experiment stations where the field conditions are controlled and crop production is backed up by latest researches from experts. The gap between this experiment station yield and the “progressive” farmers’ yield is yield gap 2. Next are the record yields which are achieved under favorable field and weather conditions by superior farmers or experiment stations. These yields which are often quite high represent the upper limits of productivity under field conditions using the latest technology. The highest possible yield of various crops is the theoretical yield which is calculated based on the photosynthetic potential and the total growth period of the crop, assuming near ideal soil conditions and nutrient use efficiency.

Average national yield of major crops in the DCs is much below the yield obtained by “progressive” farmers under similar agro-ecological conditions. The difference is represented by yield gap 1 in Figure 1. The major challenge before food-deficit countries is to bridge this yield gap as expeditiously as possible. This can be done by enabling the farmers, especially the resource poor small farmers, to implement the package of improved technology that is already being used by some of their more resourceful colleagues within the country. Availability of improved technology is obviously not the constraints in bridging this yield gap. Assured timely supply of quality inputs at affordable prices and farmer education in the proper use of these inputs is the key to bridging this yield gap. Agricultural and rural development policies especially rural roads, availability of credit, assured power supply, agriculture marketing system and pricing policy for inputs and outputs are some of the prerequisites for achieving a breakthrough in yields of major crops for which the packages of production technology have been developed and farm tested and are already being successfully used by the “progressive” farmers.

The difference between the yields of progressive farmers and experiment stations (yield gap 2) is because of the controlled conditions on the experimental fields of the research stations and availability of sophisticated facilities and technological improvements that have still not been released to farmers. As farmers get more sophisticated and the availability of machinery and farm inputs etc. is improved, this yield gap will be reduced, although there will always be some gap between the farmer and research station yields.

### **The ‘Knowledge System’**

The foundation of science-based agriculture is the knowledge system consisting of agriculture education, research and extension. Quality and relevance of agricultural education to the local agronomic and socioeconomic conditions is a critical factor in developing appropriate knowledge to improve agricultural productivity. Major developments occurred in many DCs especially in Asia in the wake of the green revolution when agricultural colleges were upgraded and new ones created to undertake research and meet the needs for additional trained personnel to man the expanded research and extension systems and the other support

services like agricultural credit institutions and input supplies etc. However the support to these institutions both from multinational and national sources has declined during the last 15 years so that many agricultural universities are now languishing due to lack of financial support which is barely sufficient to meet the salary bill for the staff with virtually no funds for faculty and student research. This has resulted in deterioration of the quality of education and generation of new knowledge to improve national agriculture. Besides, excessive reliance on the developed countries for training in applied agriculture and basing the curricula on those of the developed country institutions distorts the focus of training in agriculture making it less relevant to the agro-ecological and socio-economic environment of DCs.

Well organized national agricultural research is the key to achieving sustainable agricultural development and achieving food security. Investment in agricultural research in several DCs during the decades of 1960's and 70's produced excellent results in terms of increasing agricultural productivity and farmers' net incomes. However, there has been a gradual reduction in support of agricultural research by the countries as well as by the donor community at the bilateral and multilateral level. Donor support to agriculture declined from about 22% to 14% of all external assistance to developing countries during the 1980's<sup>o</sup>. This has detrimental effects on agricultural development and food security in the developing countries which in turn will result in increased poverty, malnutrition and pollution. These developments will run counter to the international efforts to improve the global environment for which donor assistance is being increasingly diverted.

### **Reduction of Food Losses**

Improved knowledge system resulting in a more efficient and equitable utilization of the available natural resources for agriculture undoubtedly results in increased agricultural production thereby improving the food security situation of the developing countries. However the primary production of major food crops is still prone to sizable losses during growth, harvesting, storage and post-harvest preparation. Therefore there will still be malnutrition and hunger in spite of increased primary production of food crops if the staggering losses are not reduced. This aspect is the subject of this symposium and I do hope that new approaches to reduce food losses under the conditions prevalent in most DCs, through improved food processing and preservation techniques will be discussed and practically recommendations arrived at which can be adopted by various countries.

Losses to standing crops through weeds, insects, diseases and vertebrate pests can be staggering and add up to as much as 50 to 100%. Reduction of these losses through R&D efforts is largely the responsibility of the crop experts and agriculture officials and presumably beyond the scope of this seminar. However harvesting losses of wheat and rice due to shattering, post-harvest losses in the field, and storage losses are also sizeable and need to be reduced in order to make

maximum amount of the crop produced to become available for human consumption thereby improving the food security situation. Research on improved harvesting techniques and post-harvest storage and processing therefore has to be assigned high priority. It must be pointed out that although there is a modest research effort in post harvest processing and food technology in some DCs, there is little research on these subjects in the prestigious international agricultural research centers of the CGIAR which are largely devoted to increasing agricultural production (a notable exception is IRRRI where a research group on agricultural machinery that inter alia deals with mechanization of rice harvesting). The payoff from such research is likely to be very high in view of the huge losses to the crop in the field and during storage and processing and will make a major contribution towards achievement of the cherished goal of ensuring adequate food for all inhabitants of this earth for a healthier and happier world

### **Concluding Remarks**

Developing countries need to increase their indigenous agricultural production especially of the main cereal crops to ensure adequate measure of food security for their rapidly increasing population. This is a formidable task in light of shrinking land and water resources and increasing population. If agricultural development in the food deficit countries continues at the present rates, they are bound to run into a hopeless situation regarding food availability and economic viability. However, the potential of their agricultural resources is quite high and if improved technology and appropriate policies are used, then the rate of agricultural production can be substantially increased to more than the population increase. Many currently food-deficit countries have the potential to become self-sufficient in major food grains and even become net exporters if the huge gap between average farmers' yield and that of the "progressive" farmers can be narrowed. This requires determined efforts to improve the knowledge system especially agricultural research to develop appropriate, farm-tested technology and its transfer to the farmers to achieve sustainable and efficient agriculture production. Besides a major increase in overall production of food crops, increased efforts have to be made to reduce food losses in the field and during storage and improve quality for the consumer through post-harvest preservation and processing. This requires a major strengthening of national research capabilities in food preservation and processing and adequate emphasis in the international agricultural research centers to reduce crop losses in the field due to weeds, insects, diseases and rodents etc, harvesting by traditional methods and post-harvest storage. Research in these aspects will result in more food becoming available for human consumption and a vastly improved food security especially for the poor and vulnerable groups.

**REFERENCES:**

1. Reutlinger, S. 1987. Food Security and Poverty in Developing Countries in Food Policy: Integrating Supply Distribution and Consumption. ed. J. P. Gittinger, J. Leslie, and C. Hoisington, Johns Hopkins University Press 1987.
2. Siamwalla, A. and Valdes A. 1984. Food Security in developing countries in Agricultural Development in the Third World. ed. C. K. Eicher and J. H. Staatz. Johns Hopkins University Press 1984.
3. Srinivasan, K. 1994. Critical factors affecting population growth in developing Population-the complex reality: ed. F. Graham-Smith Royal Society of London 1994.
4. Muhammed, Amir. 1992. Population and agricultural resources of the developing countries. Paper presented at the Study Week on "Population and Resources" organized by the Pontifical Academy of Sciences, Vatican City, November 17-22, 1991.
5. Food and Agriculture Organization of the UN. 1984. Land, Food and People. FAO Rome.
6. Bonte-Friedheim, Christian. 1994. Challenges, opportunities and prospects for Agricultural Research on the threshold of 21st Century - Paper presented at the First world conference of Professional in Agronomy, Santiago, Chile September 6, 1994.
7. Food and Agriculture Organization of the UN 1993. Agriculture: Towards 2010. Chapter 1-Introduction and Overview. Document C93/24 of the 27th session of FAO Conference. Rome 6-25 November, 1993.
8. UNCED Agenda 21. 1992. Promoting Sustainable Agriculture and Rural Development. United Nations Conference on Environment and Development. Rio De Janeiro June 1992.
9. Pinstруп-Andersen, P and Rajul Pandya-Lorch. 1994. Alleviating Poverty, Intensifying Agriculture, and Effectively Managing Natural Resources. Food, agriculture and environment discussion paper 1, August 1994 IFPRI, Washington D. C.
10. Plucknett, D. L. 1993. Science and agricultural transformation. IFPRI lecture series Sept. 9, 1993. IFPRI, Washington D. C.

**Table 1. International agricultural research centers of CGIAR.**

<i>Institute</i>	<i>Year of Establishment</i>	<i>Location</i>	<i>Main Focus</i>
● <b>IRRI</b> (International Rice Research Institute)	1960	Manila, Philippines	● Rice
● <b>CIMMYT</b> (Centro Internacional de Mejoramiento de Maiz y Trigo)	1966	Mexico, D.F. Mexico	● Maize, Wheat, Barley and Triticale.
● <b>CIAT</b> (Centro Internacional de Agricultura Tropical)	1967	Cali, Columbia	● Rice, Beans, Cassava, Forages and Pastures.
● <b>IITA</b> (International Institute of Tropical Agriculture)	1967	Ibadan, Nigeria	● Maize, Cassava, Cowpea, Plantain, Soybean, and Yam in humid and sub-humid tropics.
● <b>CIP</b> (Centro Internacional de la Papa)	1970	Lima, Peru	● Potato and Sweet Potatoes.
● <b>WARDA</b> (West Africa Rice Development Association)	1970	Cote d'Ivoire	● Rice in West Africa.
● <b>ICRISAT</b> (International Crops Research Institute for the Semi-Arid Tropics)	1972	Hyderabad, India	● Sorghum, Millet, Chickpea, Pigeonpea and Groundnut.
● <b>ILRAD</b> (International Laboratory for Research on Animal Diseases)	1973	Nairobi, Kenya	● Major livestock diseases in Sub-Saharan Africa
● <b>IPGRI</b> (International Plant Genetic Resources Institute)	1974	Rome, Italy	● Plant Genetic Resources
● <b>ILCA</b> (International Livestock Center for Africa)	1974	Adis Ababa, Ethiopia	● Livestock production in Sub-Saharan Africa

Table 2. Yield gain in rice after yield takeoff.

Country/Region	Takeoff year	Average yield kg/ha		Yield increase (kg/ha/yr)	
		1961	1990	Before takeoff	After takeoff
<b>North America</b>	<b>1961?</b>	<b>3800</b>	<b>6250</b>	<b>?</b>	<b>+81</b>
<b>Asia</b>	<b>1962</b>	<b>1800</b>	<b>3700</b>	<b>0.5</b>	<b>+65</b>
India	1968	1500	2700	0	+50
Philippines	1968	1250	2800	+7.0	+68
Indonesia	1967	1800	4400	+0.5	+111
Korea DPR	1967	4500	8200	-29.0	+195
<b>Latin America</b>	<b>1972</b>	<b>1800</b>	<b>2500</b>	<b>0</b>	<b>+40</b>
Colombia	1966	2000	4100	0	+95
Honduras	1982	1300	2500	+19.0	+148
<b>Africa</b>	<b>1983</b>	<b>1500</b>	<b>2000</b>	<b>0</b>	<b>+40</b>
Senegal	1980	1200	2200	0	+80
Cameroon	1975	600	3000	0	+113
Guinea Bissau	1984	800	2200	-30.0	241
Chad	1985	1100	3000	-21.0	+410

Source: Plucknett, D.L., IFPRI Lecture Series 1993

Table 3. Yield gain in wheat before and after yield takeoff.

Country	Takeoff year	Average Yield (Kg/ha)		Yield Increase kg/ha/yr	
		1961	1990	Before takeoff	After takeoff
United States	1950	-	2656	+2.3	+44
India	1966	800	2200	+5-10	+54
U.K	1950	-	6955	+4.5	+75
China	1961	600	3200	n.a	+91
Korea, DPR	1971	1800	4300	+20	+121
Egypt	1969	2500	5300	0	+128
Saudi Arabia	1980	1500	4900	+30	+260
Ireland	1976	3400	8200	+40	+285

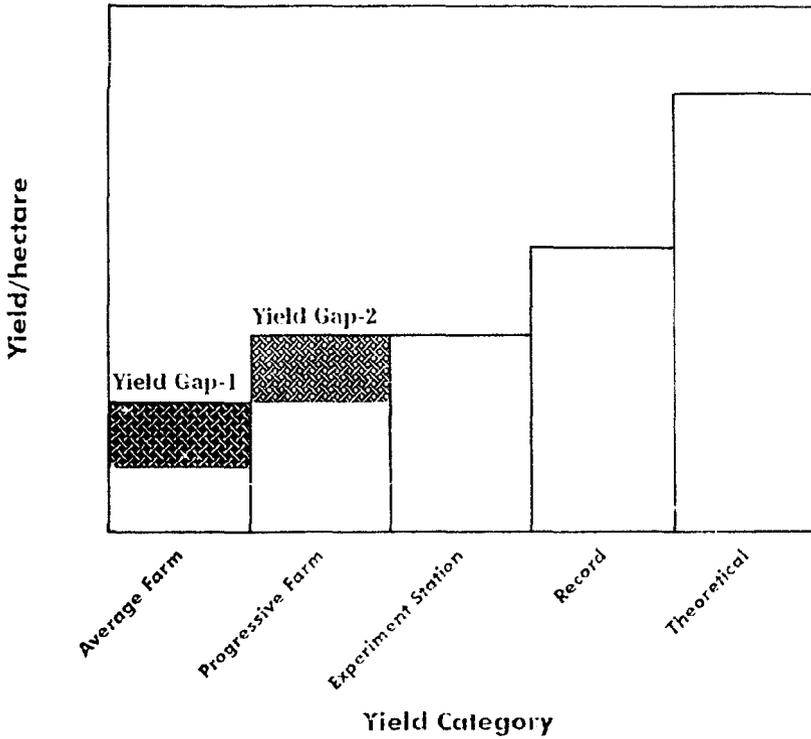
Source: Plucknett, D.L., IFPRI Lecture Series, 1993

Table 4. Yield gain in maize before and after take-off.

Country	Takeoff year	Average Yield (kg/ha)		Yield Increase kg/ha/yr	
		1961	1990	Before takeoff	After takeoff
Tanzania	1974	780	1500	0	+ 45
China	1961?	1200	4200	?	+100
Turkey	1969	1400	4000	+10	+119
United States	1937?	4000	7400	0	+126
Korea, DPR	1967	700	4200	0-2	+168
Italy	1965	3300	7600	+20	+184
Chile	1982	2000	8200	+30	+300
Niger	1987	700	1650	0	+323

Source: Plucknett, D.L., IFPRI Lecture Series, 1993

### Delineation of Yield Gaps



Source: Adapted from Plucknett D.L. (1993)

Figure 1.



**Session 1**  
**Food Safety and Security**



# MICROBIOLOGICAL CONTROL OF AFLATOXIGENIC FUNGI IN FOODS: AN EXPLORATORY STUDY

*R.C. Mabesa, M.J.V. Sumague, R.D. Divina, R.D. Lauzon,  
V. Sabariah, P.P. de Guzman, A. Villena and R.R. Melendes  
Institute of Food Science Technology  
University of the Philippines Los Baños*

## ABSTRACT

*Cladosporium fulvum* inhibited growth of *A. parasiticus* on artificial recovery media with peanut, rice and corn infusions. Inhibition was complete when *C. fulvum* was inoculated 7 day ahead of *A. parasiticus* in the infusions.

Efforts to optimize mass production of *C. fulvum* in peanut, rice and corn dextrose broths through the shake flask culture technique were undertaken. Growth in these media was slow but was rapid in malt extract broth. Consequently, *C. fulvum* was grown in this medium for mass production for toxicity evaluation.

Toxicological studies using the chick embryo bioassay showed no harmful constituent as indicated by 100% survival in the water soluble extract from medium, oil soluble extract from medium and oil soluble extract from *C. fulvum*. Likewise, the results of the mice assay also confirm that *C. fulvum* was non-toxic. No difference was noted in the physical condition of both *C. fulvum* treated and untreated mice. Histological examination revealed no apparent symptom of toxicity in all mice evaluated.

Determination of the mechanism of inhibition of *C. fulvum* was also conducted. Growth of *Aspergillus parasiticus* in potato dextrose agar adversely affected by the culture filtrate and concentrated pigment from *C. fulvum*. Morphological changes include thinning and deformation of mycelium and reduction of spore count and size.

No incidence of *A. parasiticus* growth was noted on rice treated with heated *C. fulvum* culture broth at moisture content of  $\leq 10.90\%$  even after 2 months of storage at room temperature. Likewise, the same was true for corn and peanuts with the same treatment but with moisture content of  $\leq 14.79\%$  and  $9\%$  and below respectively. No growth was observed even on the seeded rice grains, corn kernels and peanuts indicating that the inhibition was due to the treatment applied and not because of the low moisture content of the substrate.

Smoked herring (*Sardinella* sp.) treated with 100% *C. fulvum* culture broth had the lowest mold count during the first day of storage. However, it gradually increased from 7 to 35 days.

*Cladosporium fulvum* culture broth changes the natural color and odor of rice, corn, peanuts and smoked herring. It is recommended that further studies should be conducted to removed the undesirable color and odor imparted by the culture broth.

Isolation and characterization of the inhibitory compound indicated that the compound appeared to have 274 as its molecular weight and  $C_{18}H_{26}O_2$  as its tentative molecular formula.

## INTRODUCTION

One of the serious problems that confront the agricultural industry today is the postharvest handling and proper storage of agricultural commodities. The main adverse effect of improper postharvest handling and poor storage of these commodities is the loss in quality due to attack of pests, rodents and destructive microorganisms.

IDRC (1976) reported that in the Philippines the predominant microorganisms associated with stored products are fungi belonging to the *Aspergillus* group. This group of fungi is hazardous in the tropics where temperature and relative humidity are high. This is because some species of *Aspergillus* produce mycotoxins like aflatoxin which is a potent carcinogen. Peanut and peanut products cereals such as rice and corn are the principal commodities susceptible to fungal contamination and attendant aflatoxin formation. These commodities are commonly harvested with 18-20% moisture content which is ideal for mold growth and aflatoxin elaboration (Lillejoh, 1973). Smoked fish is another susceptible product because of its moisture content.

Aflatoxin is a generic term used to designate a group of highly toxic and carcinogenic metabolites produced by certain species of *Aspergillus*. It is toxic to a wide range of organisms from bacteria, to vertebrates. Production of aflatoxin is favored by warm and high humidity weather conditions which is typical of tropical regions like the Philippines (CAST, 1979). Although these molds do not attack actively growing plants, minute spores may contaminate the outer layer of the grains. If these commodities are not properly dried soon after harvest, spores germinate and multiply rapidly.

Because of the potential danger of aflatoxin to health, inhibition of toxin-producing microorganisms by chemical means, has been explored with some success, but the process is not feasible at farm level. Furthermore, the safety of the chemical treatment also needs to be assessed. Controlling relative humidity and temperature storage is another method of controlling mold growth but construction of such facility is often beyond (IDRC, 1976) what Filipino farmers can afford. Other means of suppressing mold proliferation must be sought.

The biological or microbial control approach has been considered recently as an alternative to the problems but many questions as to its efficacy and safety remain to be explored. The present research effort aims to partially resolve some issues related to the control of aflatoxin producing in food particularly in peanut, rice and corn. Briefly, the approach involves the use of other microorganisms to inhibit the growth of toxigenic fungi and thus prevent toxin formation.

## OBJECTIVE

To control the growth of *Aspergillus parasiticus* in foods using other fungi.

## METHODOLOGY

### Isolation, Screening and Identification of Potential Microorganisms.

Two hundred fifty two microorganisms representing yeasts and molds from soil, water and air were screened for their ability to inhibit *A. parasiticus*. Water samples were taken from different sources like lakes, swamps, stagnant waters and canals, while soil sample were taken gardens/plantations and from areas wherein growth of different plants are abundant.

Isolation of yeast and molds was done by plating 1 ml and 0.1 ml portions of 1/10,000 and 1/1,000,000 dilutions of soil and water in potato dextrose agar (PDA) plates acidified with 10% tartaric acid. Yeasts and molds from air were isolated by exposing PDA plates in air for 15 minutes. Plates were incubated at 32°C for 7 days. Molds were further streaked on acidified PDA plates, 3 to 4 times until a pure culture was obtained. These were maintained in PDA slants at room temperature.

A modified flask assay method by Ciegler *et al.*, (1966) was used in the pre-enrichment of isolated molds. A loopful of the test organism was transferred to an Erlenmeyer flask containing 10 ml Malt Extract Broth (MEB). The same procedure was applied to *A. parasiticus*. Both flasks containing *A. parasiticus* and the test organisms were incubated in a rotary shaker (100 rpm) at 30-32°C for 48 hours.

Equal portions (1 ml) were obtained from each flask and then mixed in a sterile petri plate. Seven to eight ml PDA medium was added to the petri plate and was allowed to solidify. Plates were incubated at 32°C for 5-7 days. Growth of both organisms and formation of zone of inhibition were observed.

The agar block technique was used in morphological examination and identification of the isolates were based on the descriptions of de Vries (1967).

Furthermore, the identified fungus with characteristics that resembles *Cladosporium fulvum* was plated with *A. parasiticum* on potato dextrose agar plates at varying inoculation periods. Both organisms were inoculated at the same time and in subsequent studies the former was given a headstart of 3, 5 and 7 days before inoculation of *A. parasiticus*.

### Determination of inhibitory Potential

To test the inhibitory potential of *C. fulvum* againsts *A. parasiticus* in peanut, rice and corn, the original medium i.ee. potato dextrose agar was modified. using the same formulation, peanut, rice and corn extracts or infusions were substituted for potato. The media were poured in petri plates and allowed to solidify. Four sets of plates were prepared. One set was inoculated with the said organisms at the same time. On the second, third and fourth sets of plates, *C. fulvum*

was inoculated 3, 5 and 7 days respectively ahead of *A. parasiticus*. Plates were incubated at 32°C for 3-5 days followed by daily monitoring of the reactions.

### **Optimization of Media Composition for the Maximum Growth of *C. fulvum***

Mass production of *C. fulvum* is necessary for toxicological studies. The organism should be evaluated for safety before it could be fully utilized against aflatoxin formers. In order to do this a substantial amount of the organism has to be grown in suitable media.

Different media were tested for the maximum growth of *C. fulvum*. These were peanut, rice and corn dextrose broths using the shake-flask culture technique for its mass production. Molisch reagent was used to monitor the presence of carbohydrates in the media. The organism was also grown in glucose and malt extracts. Each carbon source was combined with various nitrogen sources such as potassium nitrate, ammonium sulfate, peptone and urea. Ratio of carbon source to nitrogen source was 2:1. The media (100 ml each) in 125 ml Erlenmeyer flasks were shaken at 125 rpm for 2 weeks. Cells were collected through qualitative filter paper and dried at 80°C.

Labrousse and Sarejanni (1930) reported that *C. fulvum* grew abundantly in saccharides other than polysaccharides. To confirm this different carbon sources were tested for the organism such as saccharose, peanut, rice, corn and malt extract, using formulation recommended by the aforementioned investigators in their study of the genus *Cladosporium* in which *C. fulvum* was one of the species studied. Potassium nitrate was the nitrogen source used. Volume of growth medium per 125 ml flask was 100 ml. Harvest was filtered through qualitative filter paper and dried at 80°.

### **Toxicological Test**

#### **Chick-embryo assay**

For toxicity test water soluble and oil soluble extracts were obtained from dried and powdered *C. fulvum* those from the medium used for mass production of *C. fulvum*. A total of seven treatments were made namely, water soluble *C. fulvum*, oil soluble *C. fulvum*, water soluble medium, oil soluble medium, sterile distilled water control, corn oil control and egg control.

Chick embryo biological assay method was used to detect the presence of toxic substances in *C. fulvum*. A total of 210 day old chicken eggs were used and incubated in an automated incubator for 5 days. The eggs were candled to screen for live embryo and those with dead embryo and non-embryonated eggs were discarded. Those with live embryos were marked for air cells and only these marked portions were surface-sterilized with 95% ethanol. Using a flame sterilized dissecting needle, a hole was made within the area of the air cell and 0.1 ml

of either extract or control was injected and the hole was sealed using sterile melted paraffin. The eggs were labelled accordingly and reincubated in the automated incubator for 4 days. Candling was done after incubation to determine the number of live embryos.

To further determine the toxicity of *C. fulvum*, white mice assay was conducted employing the methods of DAS-VM VISCA. Seven groups (10 each group) of one month old female strong albino mice were used. Seven groups were treated with the isolate and one group served as control. Serial dilutions of  $10^1$ ,  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$  and  $10^6$  were carefully prepared from the 7-day old culture of *C. fulvum*. One ml of each dilution was injected intraperitoneally to the different groups of white mice. The assay was carried out for 7 days and daily observation was done to determine any apparent symptoms of toxicity. After the due date, the animals were sacrificed and sections of the liver tissues were collected and histologically examined for any possible effect of toxin.

### Studies on Possible Modes of Inhibition

Several dilutions from week old culture filtrate, water and oil soluble extracts from *C. fulvum* and pigment extracted from culture filtrates were carefully prepared. One ml of each dilution was thoroughly mixed with 10 ml melted sterile culture media and allowed to solidify. Conidial suspension from 5 days oil cultures of *A. parasiticus* was streaked on the solidified media and allowed to stand for 1-2 minutes to allow penetration of conidial suspension on to the culture media. Agar block was cut and transferred into sterile slides inside the petri plates and incubated at 32°. Observations were done as soon as growth sets in. Specifically, morphological changes in *A. parasiticus* were evaluated and recorded.

### Application of *C. fulvum* Culture Broth on Rice Grains, Corn Kernels and Peanuts for Storage

*Cladosporium fulvum* culture broth heated to 70° for 10 minutes was poured over the whole batch of rice and immediately drained. The treated rice was divided into 20 g portions and dried for 3, 4, 5, 6, 7 and 8 hours. Moisture content of the rice samples was determined. The same procedure was followed for corn except that it was soaked in the culture broth for 20 minutes and dried for 1, 2, 3, 4, 5 and 6 hours. For peanuts, soaking of the kernel in the culture broth was for 20 min. Another set of samples of rice grains, corn kernels and peanut were soaked in *A. parasiticus* spores suspension for 5 min (for rice and corn) and 20 min (for peanuts), respectively. These were mixed with the corresponding batch treated with *C. fulvum* culture. The samples were incubated at 32°C and growth of *A. parasiticus* monitored.

### Application of *C. fulvum* Culture Broth in Fish

Five kilos of fresh herring (*Sardinella* sp.) obtained from Lucena Fish Port were divided into 5 lots of 1 kg each. These were soaked for 30 min in 15% brine with different concentrations of *C. fulvum* culture broth (not heated). The different treatment were:

- TO - 1000 ml 15% brine (control)
- T1 - 750 ml 15% brine + 250 ml *C. fulvum* culture broth
- T2 - 500 ml 15% brine + 500 ml *C. fulvum* culture broth
- T3 - 250 ml 15% brine + 750 ml *C. fulvum* culture broth
- T4 - 1000 ml *C. fulvum* culture broth

After soaking, the fish was arranged in bamboo smoking trays, drained, sundried for 30 min and smoked for 5-6 hrs at 40°C. The smoked fish was stored at room temperature for 35 days. During storage the total fungal count, moisture and salt content of the smoked fish were monitored.

### Isolation and Partial Characterization of the Inhibitory Metabolite

Culture broth was acidified and mixed with 2% activated charcoal with overnight standing. The active compound was extracted from the filtered charcoal with methanol or acetone and the extracts were dried, extracted again with hexane and redried. The residue was loaded in a column of Sephadex LH 20. Active fractions determined by HPLC and bioassay with aflatoxigenic fungi were concentrated to dryness and further purified by HPLC. Active peak was isolated, concentrated to dryness and the residue recrystallized from methanol.

The isolated colorless residue was characterized based on its ultraviolet (UV), infra red (IR), nuclear magnetic resonance (NMR) and mass spectra.

## RESULTS AND DISCUSSION

### Isolation, Screening and Identification of Potential Microorganisms

Results showed that out of 252 isolates tested, only one was truly capable of inhibiting the growth of *A. parasiticus* completely. A few strains of *A. niger* group were able to outgrow *A. parasiticus* initially but was outgrown on extended incubation. Fungal isolate from air identified as *A. fumigatus* yielded positive results in restricting the growth of *A. parasiticus* but potential toxicity limits its application in food.

A fungal isolate from soil with characteristics typical of *Cladosporium*

*fulvum* (Figures 1a, 1b and Table 1a, 1b) showed excellent antagonistic characteristics because it inhibited *A. parasiticus* on potato dextrose agar (Figure 2). Furthermore, when *C. fulvum* was given a headstart on potato dextrose agar complete suppression of *A. parasiticum* was observed (Figure 3).

### Evaluating the Inhibitory Potential of *C. fulvum*

Complete inhibition was evident when *C. fulvum* was allowed to grow 7 days ahead of *A. parasiticus* (Figure 4). The longer the lead time the better the inhibition. No inhibition was observed on the plates where the organisms were grown at the same time.

Partial inhibition was observed in the plates where *C. fulvum* was inoculated 3 and 5 days ahead of *A. parasiticus*. The growth of *A. parasiticus* was limited only in the nonpigmented area of the culture media. The pigment produced by the organism may be the inhibitory component which restricted growth of *A. parasiticus*. Some microorganisms produced pigmented compounds for their survival and often times these substances were inhibitory to other microorganisms.

### Optimizing Media Conditions for Maximum Growth of *C. fulvum*

Growth of *C. fulvum* in peanut, rice dextrose broth and corn dextrose broths was very slow. The media were not utilized fully by the organism. Molisch test showed that carbohydrates were still present even after 3 weeks of shaking. In view of this other media were tested to determine the optimum medium for the organism.

Other media tested were malt extract and glucose with different nitrogen sources. Growth was observed in malt extract with ammonium sulfate, potassium nitrate and peptone. This was confirmed by a change of the original color of the substrate from yellow to purple or maroon. No growth was observed in the medium with urea as nitrogen source. In glucose, the only medium which yielded growth was the one with potassium nitrate as nitrogen source (Table 2). Dry cell weight was highest in malt extract with peptone as nitrogen source. In subsequent experiments however potassium nitrate was selected since it was less expensive (P895/500g) than peptone (P2,200/454g). From among the carbon sources studied malt extract gave the highest dry cell weight (Table 3), therefore it was chosen for the mass production of *C. fulvum*. The formulation by Labrouse and Sarejanni (1930) was utilized for the said purpose.

### Toxicological Test

#### Chick-embryo assay

The toxicological test using chick embryo bioassay (Table 4) shows 100%

survival in the water soluble extract from medium, oil soluble extract from medium and oil soluble extract from *C. fulvum*. This indicates that there were no toxic substances present in the extracts. On the other hand, 97% survival was obtained from distilled water control, water soluble extract *C. fulvum* and egg control and 80% survival from the oil control. Death of chick embryos in these treatments were believed to be due to the physical condition of the chick embryos themselves and not because of toxic substances considering that no mortality occurred in both oil-soluble extract from *C. fulvum* and water soluble extract from medium. The occurrence of 3% mortality in the control treatment (uninjected eggs) reinforces that some deaths were attributed to the chick embryos.

Death of the chick embryos in the oil control may be due to some substances like antioxidant present in the oil which might be detrimental to the sensitive embryos. These observations conform to the findings of Villalalvo (1988) where chick embryos inoculated with high grade cooking oil yielded only 90.49% survival. In this particular treatment 80% survival still fall on the normal level of fertility rate of the hatching eggs of the Alabang Experimental Station (Santiago, 1991)

#### White Mice Assay

After 7 days of intraperitoneal injection with *C. fulvum* suspension, physical conditions of the experimental animals was found normal and resembled that of the control group. Result from histological examination revealed no apparent symptom of toxicity in all groups of animals evaluated. This result is in agreement with the findings of Scott (1984) who reported that neither *Cladosporium* nor related compounds are proven to be toxic. Mirocha as pointed out by McLean (1971), further supports the results of this study by reporting that no estrogenic activity or other apparent toxic action was noted in weaning rate when a 2 mg total dose of *Cladosporium* was given for over 5 days. Scott and Walbeek (1974) further reported that *Cladosporium* was non-toxic to mice at dose of 400 mg by intraperitoneal injection. These finding confirm that *Cladosporium fulvum* is a non-toxic fungus.

#### Possible Modes of Inhibition.

The inhibitory activity of *C. fulvum* against *A. parasiticus* may be due to still unidentified active compound produced by the former which alter the morphology of the latter. When *A. parasiticus* was plated on potato dextrose agar with freshly collected culture filtrate the hyphae were deformed and were reduced in size (Fig. 5). The same results were observed when water soluble, oil soluble and pigment extracted at neutral condition were used except that enlargement of some portion of the mycelium were noted in *A. parasiticus* plated with

the pigment (Table 5). When *A. parasiticus* was plated with pigment extracted at acidic and alkaline condition, different morphological deformations were noted (Fig. 6). Some hyphae contracted or shortened in length while others disintegrated. Less spores were also produced and spore size were smaller than untreated ones (Fig. 7 and 8). Apparently spore germination was also impaired, Weindling (1941) also found that the presence of an antagonistic fungus *Trichoderma* resulted in the disintegration of hyphae of the plant pathogenic fungus *Rhizoctonia*. Borodulina (1945) reported that antibiotic substances produced by actinomycetes affected the morphology of *B. mycoides* by cell elongation, cell enlargement and reaching to an abnormal size and assuming the most peculiar forms and delayed spore formation. The overall effect of antagonistic substances produced by fungi comprise the changes in forms, size, structure of hyphae, direction of growth and abbreviation of hyphal structure (Walksman and Belly, 1945).

#### **Application of *C. fulvum* Culture Broth on Rice, Corn and Peanuts for Storage.**

The culture broth was used in actual application experiments on rice, corn and peanuts. Preliminary results showed that the culture broth had to be heated to 70°C for 10 minutes to prevent growth of *C. fulvum* on the samples. Heating did not affect the inhibitory ability of the culture broth. The heated culture broth still showed its inhibition in the agar cup test of determining zone of inhibition which indicated that it was heat stable (Table 6).

No incidence of *A. parasiticus* growth was noted on rice at moisture content of < 10.90% and on corn at moisture content of > 14.79% even after 2 months of storage (Table 7). This implies that at the said treatment *A. parasiticus* cannot grow on the samples. No growth was found even on the seeded rice grains and corn kernels. The results show that the filtrate was very effective in inhibiting the growth of *A. parasiticus*. In the preliminary experiments, however, rice with > 19.37% and corn with > 21.94% moisture had *A. parasiticus* growth.

Results showed that growth of *A. parasiticus* in peanut was suppressed for a period of 6 days only when the moisture content was above 9%. However, if the moisture content was maintained at a 9% and below, complete inhibition of growth of *A. parasiticus* was noted even at prolonged storage (more than 3 months) at room temperature. Likewise, no visible growth of *A. parasiticus* was observed in the seeded peanut (soaked in *A. parasiticus* spore suspension for 20 min.) indicating that the inhibition was due to the treatment applied (*C. fulvum*) are not because of the low moisture content of the substrate.

However, there is one limitation in the use of the culture filtrate because it altered the color of rice, corn and peanuts to violet. In the future, further trials should be conducted on a larger scale to determine the effectivity of the culture broth on larger volume of produce.

### Application of *C. fulvum* Culture Broth in Fish

Increasing levels of culture broth resulted in substantial reduction of mold count in the treated smoked fish up to 7 days of storage (Fig. 9 and 10). However, after this period, the counts started to rise in treated samples compared to the control. It appears that the inhibitory substance elucidated by *C. fulvum* had lost its inhibitory properties after 7 days. Jarvis (1971) reported that some metabolites lose this property after 8 days under laboratory conditions. This loss was brought about by some strains of microorganisms which degrade metabolites.

*Aspergillus*, *Cladosporium*, *Helminthosporium*, and *Mucor* were the genera of the molds found in the smoked fish samples. It is possible also that these genera of molds were partially responsible for the loss of inhibitory activity of the culture broth. However, this requires further verification in future studies.

The overall sensory quality of smoked fish treated with brine was generally acceptable. However, the color and odor of the smoked fish treated with different concentrations of *C. fulvum* culture broth became offensive as the storage period increased.

### Characterization of the Isolated Metabolite

The ultra violet spectrum of the active compound had maximum absorption at 261.5 nm inferring the presence of chromophoric group(s). Main absorption bands of the infrared spectrum appeared at wavelengths 3300, 3800-3000 and 1651  $\text{cm}^{-1}$ . The compound has -OH groups but no carbonyl functionality. The nuclear magnetic resonance (NMR) spectrum ( $\text{CDCl}_3$ ) was assigned as follows  $\text{CH}_3$ - at 1.58 (6M, br s),  $\text{CH}_3$ - at 1.68 (3H, br s), ---  $\text{CH}_2$ - at 1.90 2.30 (1H,m), -OH at 4.90 5.90 (2H, br), C=C-H at 5.10 (3H, br s) and -OH at 5.72 (1H, br s). The mass spectra showed that the mass unit of the parent ion is 274. Integration of the total number of protons using the NMR spectrum suggested 26 hydrogen atoms for the compound. Assuming that it contains carbon and 2 oxygen atoms, the calculated molecular weight is 274.404. Therefore the compound appeared to have 274 as its molecular weight and  $\text{C}_{10}\text{H}_{26}\text{O}_2$  as its tentative molecular formula. The available data did not allow full elucidation of the compound.

### SUMMARY

Inhibitory potential of *C. fulvum* against *A. parasiticus* in peanut, rice and corn infusions was evaluated. Results indicate that inhibition increased as lead time for the inhibitory fungus (*C. fulvum*) increased. Complete suppression was observed when *C. fulvum* was inoculated 7 days ahead of *A. parasiticus* in the infusions.

Furthermore, optimization of the growth of *C. fulvum* through shake flask culture for mass production was also conducted. Growth of the said organism in

peanut, rice and corn dextrose broths was very slow. Malt extract broth yielded vigorous growth and was consequently used for the mass production of *C. fulvum* for toxicological evaluation.

*Cladosporium fulvum* was found to be nontoxic. Toxicological evaluation showed 100% survival in water and oil soluble extracts from medium and oil soluble extract from *C. fulvum*. The non toxicity of *C. fulvum* was further confirmed by the mice assay.

Experiments on the possible effect of *C. fulvum* on *A. parasiticus* show that the culture filtrate and pigment from *C. fulvum* maybe largely responsible for the inhibition of *A. parasiticus*. Microscopy revealed that these substances caused the thinning of cell walls of the fungus and deformation of the hypha and reduction in the size and number of spores. Germination was likewise adversely affected.

No incidence of *A. parasiticus* growth was noted on rice treated with heated *C. fulvum* culture broth at moisture content of < 10.90% even after 2 months of storage at room temperature. Likewise, the same was true for corn and peanuts with the same treatment but with moisture content of <14.79% and below respectively. No growth was observed even on the seeded rice grains, corn kernels and peanuts indicating that the inhibition was due to the treatment applied and not because of the low moisture content of the substrate.

Smoked herring (*Sardinella sp.*) treated with 100% *C. fulvum* culture broth had the lowest mold count during the first day of storage. *Cladosporium fulvum* culture broth actively reduced total mold count on smoked fish for only a week. Mold count gradually increased from 7 to 35 days. Smoked fish treated with 15% brine only had the lowest count after 14 days up to 35 days of storage at room temperature due to its low moisture content, high salt content and the absence of *C. fulvum* culture broth.

*Aspergillus*, *Mucor*, *Helminthosporium* and *Cladosporium fulvum* culture broth because it changed the natural color and odor of rice, corn, peanuts and smoked herring. It is recommended that further studies should be conducted to find a solution to the undesirable color and odor brought about by the culture broth.

Further studies show that that inhibitory compound was not the pigment formed by *C. fulvum* but a substance closely associated with the pigment.

Isolation and characterization of the inhibitory compound indicated that the compound appeared to have 274 as its molecular weight and  $C_{18}H_{26}O_2$  as its tentative molecular formula.

**LITERATURE CITED**

- Borodulina, J.A. 1945. Interrelations of soil actinomycetes and *B. mycodes*. Microbiology. USSR. 4:561-586.
- CAST. 1979. Aflatoxin and other mycotoxins: An Agricultural perspective. Council for Agricultural Science and Technology. Report no. 80 Ames. Iowa.
- Ciegler, A., E.B. Lillejoh, R.E. Peterson and H.H. Hall. 1966. Microbial Detoxification of Aflatoxin. Applied Microbiol: 14:934-939.
- International Development Research Centre. 1976. Rice-Postharvest Technology. Edited by Auraullo, E.V., D.B. Padua and M. Graham. IDRC-C53 pp.144-149.
- Jarvis, B. 1971. Factors affecting the production of mycotoxins. J. Appl. Bact. 34(1):199-213.
- Labrouse, F. and J. Sarejanni. 1930. Recherches physiologiques sur quelques chapignons parasites. Physopathologische Zeitschrift. 2: 1-38.
- Lillejoh, E.B. 1973. Feed sources and conditions conducive to production of aflatozin, ochratoxin. Fusarium toxins and zearalenone. J.Am. Vet. Med. Assoc. 163:1281-1284.
- McLean, W. M. 1971. *Cladosporium*. A new antifungal metabolite form *Cladosporium cladosporoides*. The Journal of Antibiotics. 24(11):747-755.
- Scott, P.M. 1984. Mycotoxin Production, Isolation, Separation and Purification. V. Betana (ed) Elsevier Science Publishers, E.V. Amsterdam. Printed in Netherlands pp.457-461.
- Scott, P.M. and W.V. Walbeek. 1974. U.S. Pat. 3., 383,171, Sept 24, 1974; C.A., 83 (1975) 26271c; Can. Pat. 990, 231, June 1, 1976.
- Santiago, R. 1991. Personal communication. Officer in charge of Hatching Section of the BAI Alabang Experimental Station.
- Villalalvo, J. 1988. M.S. Thesis. Unpublished, University of the Philippines at Los Banos, College. Laguna.
- Walkmans, S.A. and H.C. Relly. 1945. The agar streak method for assaying antibiotic substances. Ind. Eng. Chem. Ana. Ed. 17:556-558.

- Vries de G.A. 1967. Contribution to the knowledge of the genus *Cladosporium* Link Ex. R. Wheldon and Weley Ltd. New York. p. 70-72.
- Weindling, R. 1941. Studies on a lethal principle effective in the parasitic action of *Trichoderma Liqueurum* on *Rhizectonia salau* and other soil fungi. *Phytophat.* 244:1153-1179.



# OCCURRENCE OF FOOD-BORNE PATHOGENS IN LAGUNA 'KESONG PUTI' AND 'PASTILLAS DE LECHE'

V. L. Barraquio, P. A. Pacanot, Ma. C. dP. Trinos, and E. N. Almazan  
Dairy Training and Research Institute (DTRI),  
College of Agriculture, University of the Philippines,  
Los Banos, Laguna, Philippines 4031

## ABSTRACT

'Kesong puti' (soft cheese) and 'pastillas de leche' (dairy confectionery) were studied for their microbiological quality and their contamination with food-borne pathogens. Higher mean microbial counts were found in 'kesong puti' with  $5.8 \times 10^6$  cfu/g total bacteria,  $4.1 \times 10^5$  cfu/g coliforms and  $5.9 \times 10^4$  cfu/g yeast and molds. *Escherichia coli* type I and *Listeria* sp. were isolated from 'kesong puti'. 'Pastillas de leche' samples had better microbiological quality with mean microbial counts of  $3.4 \times 10^3$  cfu/g total bacteria, 7 cfu/g coliforms and  $8.9 \times 10^1$  cfu/g yeast and molds. The 'pastillas de leche' samples were negative for all the food-borne pathogens investigated.

## INTRODUCTION

'Kesong puti' and 'pastillas de leche' are dairy products indigenous to the Philippines. Both are manufactured in cottage-type enterprises in certain regions of the country.

'Kesong puti' is an unripened soft cheese generally produced by coagulating the raw carabao's milk with either vinegar or abomasal extract. The coagulum is broken, then salted either before or after placing into moulds then drained for a few minutes before wrapping in banana leaves.

'Pastillas de leche' is a milk-based confectionery traditionally prepared with carabao's milk by condensing the milk with addition of sugar and stabilizer. It is a relatively soft, bar-shaped, white milk candy weighing approximately 5 to 6 grams and wrapped in white paper.

To our knowledge, no food-borne disease outbreaks have been reported yet in connection with 'kesong puti' and 'pastillas de leche' consumption in the Philippine. Although studies abroad have implicated both raw and pasteurized milk with food-borne pathogens (Moustafa 1990; Garayzabal et al. 1986; Fenlon & Wilson 1989; Stone; Slade et al 1988; Davidson et al. 1989; Tiwari & Aldenrath 1990) raw milk poses the greater danger. *Listeria* and *Versinia* sp. have been reported in various types of soft cheeses (Moustafa 1990; Wernars et al. 1991).

This study was undertaken to assess the microbiological quality of 'kesong puti' and 'pastillas de leche' produced in Laguna province and to determine the

presence of food-borne pathogens in these dairy products.

## **MATERIALS AND METHODS**

### **Sampling**

A total of 25 samples each of 'kesong puti' and 'pastillas de leche' were collected from the towns of Los Banos and Bay in Laguna province and used for the study.

### **Microbiological Examination**

Total bacterial count was carried out following I.D.F. (1987) procedure. For coliform count, the procedure described by I.D.F. (1985a) was followed while I.D.F. (1985b) method was used for the determination of yeast and mold count.

### **Chemical Analyses**

'Kesong puti' samples were analyzed for moisture and salt contents (A.O.A.C. 1990) while 'pastillas de leche' samples were analyzed for moisture only.

### **Detection of Food-borne Pathogens**

The method of Harrigan and McCance (1976) was used for the detection of *Escherichia Salmonella* and *Shigella* were determined following the procedures of Harrigan and McCance (1976) and I.D.F. (1985c). For *Yersinia* and *Staphylococcus* detection, the procedures described by BBL (1988) were followed. *Listeria* detection was carried out using the enrichment procedure by the A.P.H.A. (1992) followed by Listeria-tek ELISA assay (AKZO Pharma Division, Organon Teknika, Philcox Bldg., Legaspi St., Makati, Metro Manila, Philippines).

## **RESULTS**

### **Microbiological quality of 'kesong puti' and 'pastillas de leche'**

Higher microbial counts were obtained in 'kesong puti' than in 'pastillas de leche'. This is attributed to the higher moisture content of kesong puti'.

### Occurrence of food-borne pathogens

Of the suspected isolates from 'kesong puti' and 'pastillas de leche', 11 from 'kesong puti' were confirmed to be *Escherichia coli* type I while the rest showed reactions typical of *Enterobacter aerogenes* (Table 3).

Using Listeria-tek ELISA assay, a sample was considered presumptive positive if its absorbance is equal to or greater than the cut off absorbance value. Cut off value = negative control mean + 0.150. The computed cut off value for the samples tested was 0.599, and for the first trial, 6 'kesong puti' samples were found to be presumptive positive with absorbance values greater than 0.599. These presumptive positive samples were again tested using the same assay procedure, and the computed cut off value was 0.3495. Of the 6 presumptive positive 'kesong puti' samples, 2 were confirmed to be *Listeria* positive (Table 4). The false positive results can be attributed to insufficient washing of the wells.

No *Salmonella*, *Shigella*, *Yersinia* and *Staphylococcus* were isolated from 'kesong puti' while 'pastillas de leche' samples were negative for all the food-borne pathogens studied.

### DISCUSSION

In cheesemaking, the bacterial content of the cheese is a reflection of hygienic practices during the processing and handling of both milk and cheese. Some bacteria cause spoilage and some are pathogenic, thus a low total plate count is desirable. High bacterial content is indicative of low keeping quality of the cheese. Spoilage is indicated by proteolysis, gas production, sliminess and discoloration (Frazier 1988).

Lavapiez (1974) reported counts ranging from  $3.4 \times 10^2$  to  $5.6 \times 10^4$  cfu/g in cheese from Los Banos, Laguna. The milk used for cheesemaking by the Los Banos and Bay cheesemakers are pasteurized, therefore high bacterial counts reflect unhygienic practices during the processing and handling of both milk and cheese. Coliforms which are indicators of post pasteurization contamination comes from poorly sanitized utensils used in the preparation of cheese such as pails, cans, ladles, cheese moulds, cheese cloths, and cheese wrappers and/or from food handlers or those who prepare the cheese.

'Kesong puti' being made from milk, is rich in nutrients, rapid growth and multiplication of contaminant are expected. Furthermore, the high moisture content, averaging 73.9%, of 'kesong puti' favors the growth of most contaminants. Its salt content of 1.6% on the average, is not high enough to inhibit growth of organisms.

Although *Escherichia coli* type I and *Listeria sp.* were found in 'kesong puti' in this study, no disease outbreak from the consumption of 'kesong puti' from Laguna has been documented. It is probable that these organisms were present below the infective dose level. For pathogenic *E. coli*, large numbers, greater or equal to  $10^6$  cells are required for illness while for *Listeria* and other

etiological agents, the infective dose has yet to be determined (D'Aoust 1989). Previous studies have demonstrated the survival of *E. coli* and *L. monocytogenes* in cheeses stored at temperature ranges of 3 to 13°C (Ryser and Marth 1987; Kornacki & Marth 1982; Youssef & Marth 1988; Frank et al. 1977; Farber et al. 1987).

Overall, 'pastillas de leche' samples showed better microbiological quality than 'kesong puti' samples. This could be attributed to the long heating process involved in making 'pastillas de leche' its low moisture (7.4%, average) and high sugar contents. Although the milk used for 'pastillas de leche' making is not pasteurized, heat is applied for a long period during its preparation. The milk, sugar and stabilizer (usually cornstarch) are mixed and cooked until much of the moisture is removed and the texture becomes pasty. The prolonged heating is enough to kill the microbial load of the milk and the ingredients. The low moisture and high sugar content prevents growth of microorganisms which may contaminate after cooking.

#### ACKNOWLEDGEMENT

We are grateful to AKZO Pharma Division, Organon Teknika for the complimentary Listeria-tek ELISA kit.

#### REFERENCES:

- A.O.A.C. (1990) Official Methods of Analysis of the American Association of Official Analytical Chemists, Vol. II, 15th edn. Helrich, K. pp. 840-842. Arlington, VA: American Association of Official Analytical Chemists.
- A.P.H.A. (1992) Compendium of Methods for the Microbiological Examination of Foods, 3rd ed. Vanderzant, C. & Splittstoesser, D. F. pp 637-663. Washington, D. C.: American Public Health Association
- BBL (1988) Manual of BBL Products and Laboratory Procedures, ed. Power, D. A. & McCuen, P. J. 389 pp. Cockeysville, Maryland: Becton Dickinson Microbiology Systems.
- D'AOUST, J. -Y. (1989) Contemporary concerns on the microbiological safety of milk and dairy products. In Modern Microbiological Methods for Dairy Products, IDF Special Special Issue No. 8901, pp. 15-44. Brussels, Belgium: International Dairy Federation
- DAVIDSON R. J., SPRUNG, D.W., PARK, C. E. & RAYMAN, M. K. (1989) Occurrence of *Listeria monocytogenes*, *Campylobacter* spp. and *Yersinia*

- enterocolitica in Manitoba raw milk. Canadian Institute of Food Science and Technology Journal 22, 70-74.
- FARBER, J. M., JOHNSTON, M. A., PURVIS, U. & LOIT, A. (1987) Surveillance of soft and semi-soft cheese for the presence of *Listeria* sp. International Journal of Food Microbiology 5, 157-163.
- FENLON, D. R. & WILSON, J. (1989) The incidence of *Listeria monocytogenes* in raw milk from bulk tanks in North-East Scotland. Journal of Applied Bacteriology 66, 191-196.
- FRANK, J. F., MARTH, E. H., & OLSON, N. F. (1977) Survival of enteropathogenic and non-pathogenic *Escherichia coli* during the manufacture of Camembert cheese. Journal of Food Protection 40, 835-842.
- FRAZIER, W. C. (1988) Food Microbiology, pp. 396-400. New York: McGraw Hill Book Co.
- GARAYZABAL, J. F. F., RODRIGUEZ, L. D., BOLAND, J. A. V., CANSELO, J. L. B. & FERNANDEZ, G. S. (1986) *Listeria monocytogenes* dans le lait pasteurise. Canadian Journal of Microbiology 32, 149-150.
- HARRIGAN, W. F. & McCANCE, M. E. (1976) Laboratory Methods in Food and Dairy Microbiology, 452 pp. London & New York: Academic Press.
- I.D.F. (1985a) Enumeration of coliform-colony count technique and most probable number technique at 30°C, 73A, pp. 1-8 Brussels, Belgium: International Dairy Federation.
- I.D.F. (1985b) Detection and enumeration of yeast and molds, 94A, pp. 1-3. Brussels, Belgium: International Dairy Federation.
- I.D.F. (1985c) Detection of *Salmonella*, 93A, pp. 1-9. Brussels, Belgium: International Dairy Federation.
- I.D.F. (1987) Enumeration of microorganisms by colony count at 30°C, 100A, pp. 1-5. Brussels, Belgium: International Dairy Federation.
- KORNOCKI, J. L. & MARTH, E. H. (1982) Fate of nonpathogenic and enteropathogenic *Escherichia coli* during the manufacture of Colby-like cheese. Journal of Food Protection 45, 310-316.
- LAVAPIEZ, L. (1974) A Study of Microbiological Contamination in Cheesemaking. Undergraduate Thesis, University of the Philippines, College of Agriculture, College, Laguna, Unpublished

- MOUSTAFA, M. K. (1990) Isolation of *Yersinia enterocolitica* from raw milk and soft cheese in Assuit City. Dairy Science Abstracts.
- RYSER, E. T. & MARTH, E. H. (1987) Fate of *Listeria monocytogenes* during the manufacture and ripening of Camembert cheese. Journal of Food Protection 50, 372-378.
- SLADE, P. J., COLLINS-THOMPSON, D. L. Incidence of *Listeria* species in Ontario milk. Canadian Institute of Food Science and Technology Journal 21, 425-429.
- STONE, D. L. (1987) A survey of raw whole milk for *Campylobacter jejuni*, *Listeria monocytogenes* and *Yersinia enterocolitica*. New Zealand Journal of Dairy Science and Technology 22, 257-264.
- TIWARI, N. P. & ALDENRATH, S. G. (1990) Occurrence of *Listeria* species in food and environmental samples in Alberta. Canadian Institute of Food Science and Technology Journal 23, 109-113.
- WERNARS, K., HEUVELMAN, C. J., CHAKRABORTY, T. & NOTERMANS, S. H. (1991) Use of polymerase chain reaction for direct detection of *Listeria monocytogenes* in soft cheese. Journal of Applied Bacteriology 70, 121-126.
- YOUSSEF, A. E. & MARTH, E. H. (1988) Behavior of *Listeria monocytogenes* during the manufacture and storage of Colby cheese. Journal of Food Protection 51, 12-15.

# FRESHNESS ASSESSMENT OF FISH MEAT USING THE TORRYMETER, IMPEDANCE ANALYZER AND K VALUE

*M. Sakaguchi, A. Koike, S. Takahashi and K. Kato*  
*Faculty of Agriculture, Department of Fisheries*  
*Kyoto University, Japan*

## INTRODUCTION

Freshness is a most important factor to determine the quality of fish. Freshness of fish has so far been measured mainly using sensory methods; these methods are based on human judgements which can very subjective, although the methods have such advantages as quickness and nondestructiveness. Chemical methods, for example, depend on measuring the amounts of various compounds such as trimethylamine and histamine produced by microbial action and of ATP breakdown products (hyposanthine and inosine) by endogenous enzyme action<sup>1,2</sup>. Generally, all chemical methods have the disadvantage of being time consuming and destructive in nature. As a physical procedure to assess the freshness of fish, the Torrymeter, an electric fish freshness tester<sup>3,4</sup> is widely known. This instrument in fact has the capability to measure the freshness nondestructively at high speed.

We report here, however that the K value and Torrymeter test measures the freshness of fish (yellowtail) meat in later periods of ice storage but not in earlier periods. Furthermore, we describe the results of an experiment to evaluate the freshness of fish fillets using an impedance analyzer in the storage periods.

## MATERIALS AND METHODS

### Material

Three live yellowtail *Seriola quinqueradiata*, designated as Yt1, Yt2, and Yt3 (1.6-2.3 kg body weight) were purchased from a local market. They were decapitated and filleted immediately. One of the two fillets obtained from each fish was used for measuring K value and the other of the fillets was done for electric properties. Each fillet was packaged in a polyethylene bag and submerged in ice during storage. The bag containing the fillet was immediately sealed to prevent contamination from melted ice and submerged in ice which was replaced with fresh one as required.

### Determination of the K value

Several muscle pieces were dissected from the dorsal muscle separated from one fillet of fish. Those muscle pieces obtained from all 3 fish were pooled and mixed thoroughly to ensure the homogeneity. The pieces were submitted for

extraction with 10% (w/v) perchloric acid (PCA) according to the method described by Nakajima et al.<sup>5)</sup> Concentrations ( $\mu$  mole /g wet muscle) of ATP and its degradation products in the PCA extract were estimated by the method of Ryder.<sup>6)</sup> The K value (KV) was calculated by substituting these concentrations in the equation proposed by Saito et al.<sup>7)</sup>

### **Measurement of Torrymeter reading**

The Torrymeter reading (TMR) was taken with a GR Torrymeter (GR International Electronics) just on the lateral line (skin side) and on the dorsal part (bone side) of the skin-on fillets. The meter was used in the individual mode.

### **Analysis of impedance parameters**

Electrical properties of the fillets of various freshness were measured on an impedance analyzer according to the method described by Kato<sup>8)</sup>. Swept frequency measurements were performed from 1KHz to 1MHz and reactance as the imaginary part (-X) was plotted against series equivalent resistance ( $R_s$ ) as the real part of the impedance. Both needle type (diameter 2mm, length 20mm, with 40mm) and wet contact type electrodes (diameter 38mm) were used. Measurements using the former type electrode were carried out by piercing the dorsal part of muscle through the surface layer and with the latter type electrode by placing it on the same dorsal part of fish body.

## **RESULTS AND DISCUSSION**

### **Changes in KV during storage**

Figure 1 shows the change in KV of the yellowtail fillets during ice storage. The value was very low for the extremely fresh fillets (less than 1%) and then increased linearly until the last day of storage (ca. 40%). For the first 3 days, KV increased by 10% and reached ca.20% approximately 5 days after the beginning of ice storage. The value of 20% has been reported on tuna meat as a freshness limit of "Sashimi"<sup>9)</sup> of high quality. Our preliminary sensory observation on the yellowtail fillets, however, indicated that they had an unpalatable flavor upon storage in ice for 5 to 6 days. Therefore, the KV is considered as a freshness index of yellowtail fillets for storage periods longer than 5-6 days.

### **Changes in TMR during storage**

Figures 2 and 3 depicts the change in TMR measured on skin and bone sides of the skin-on fillets during storage, respectively. By approximately 4 days after the beginning of storage, a sharp decrease in the reading were observed for the fillets measured on bone side (Figure 3), while the reading on skin side fell slowly

especially in the earlier period of storage (Figure 2). Additionally, our previous report indicated that the reading on skin side does not always parallel the degree of freshness for such fish species as flounder and filefish.<sup>9)</sup> This evidence suggests that the measurement should be performed on the bone side, when it is required for such a material as "Sashimi" which is commonly consumed very fresh.

The Torrymeter measures the changes in dielectric properties of tissue cells after death of fish.<sup>3)</sup> This changes could be ascribed to the deterioration of cellular structure including cell membrane<sup>4)</sup>. The difference in shape of the curve between skin and bone sides (Figure 2 and 3) possibly due to the deterioration of cellular structure.

Figure 4 shows the relation between the change in KV (Figure 1) and that in TMR (Figure 2 and 3) during ice storage. It has been reported that tuna "Sashimi" of high quality has KVs of less than 20%<sup>2)</sup>. If it is true for yellowtail, this value corresponds 7 and 6 of TMR measured on skin and bone sides, respectively, suggesting that the KV, a method can be placed by TMR, a nondestructive method.

### Changes in electrical properties during storage

Electrical properties of skin-on fillets stored in ice in various periods were measured in range of frequencies from 0.1 KHz to 1MHz. Figures 5-A and 5-B depict the changes in vector impedance trajectories (Cole-Cole arcs) measured using needle type electrodes on skin and bone sides, respectively, during storage. Figures 6-A and 6-B also show the changes measured using wet contact type electrodes on skin and bone sides, respectively. When the fillets were aged, the arcs rapidly shrank except for the samples measured on skin side using the wet type electrode. This phenomenon has been known on some fruits, e.g. apples and pears, as reported by Kato<sup>8)</sup>.

The changes in radius of the arc measured on bone side using needle type (Figure 5-B) and wet contact type electrodes (Figure 6-B) are shown in Figure 7-A and 7-B, respectively. The extent of change measured on bone side was greater than that measured on skin side (data not shown). The radius fell rapidly to lower levels 10 to 20 hours after the start of storage; the smaller the radius became, the lower levels the freshness reached, suggesting that the measurement of radius is applicable to estimating the quality of fish for "Sashimi" which require extremely high freshness.

The impedance analyzer is also able to measure the difference in series equivalent resistance (Rs) between two definite frequencies. Figures 8-A and 8-B show the changes of difference in Rs between 10K and 1MHz, which were analyzed on bone side using needle type and wet contact type electrodes, respectively, during storage. The difference in the Rs decreased sharply to lower levels as early as about 20 hours, also suggesting that this measurement is able to estimate the eating quality of fish meat for products such as "Sashimi". The significant decrease in muscle breaking strength, an index of "Sashimi" texture, was

reported to start shortly after the beginning of storage and ceased at approximately 24 hours.<sup>10</sup> At this period of storage, the KV was still low (less than 5%) (Figure 1) and the TMR was as high as 10-13 (Figures 2 and 3). It was concluded that the impedance analyzer is applicable to determine the quality of fish meat which require very high freshness.

Skin-on fillet prepared from very fresh yellowtail were stored in ice and examined for freshness nondestructively using the Torrymeter and impedance analyzer. The K value a freshness index was also measured on HPLC in a destructive manner. Within the range of storage period employed up to 12 days, the Torrymeter reading measured on bone side fell rapidly in earlier periods of storage (0-3 days), while the reading on skin side decreased slowly. The K value increased gradually up to 35-40% at the end of storage. The analysis of impedance parameters for the fillets of various degrees of freshness indicated that the radius of Cole-Cole arc and the difference in series equivalent resistance between 10KHz and 1MHz decreased rapidly by about 20 hours after the beginning of storage. Mostly, their rates of decrease were much higher than those of the Torrymeter reading and K value, suggesting that the impedance analyzer is applicable to measuring the quality of fish meat of high freshness.

## REFERENCES

- 1) Z. E. Sikorski, A. Kolakowska and J. R. Burt: Postharvest biochemical and microbial changes. *Seafood: Resources, Nutritional Composition, and Preservation* (Z. E. Sikorski ed.), 1990, CRC Press, Boca Ranton, pp. 55-57.
- 2) S. Ehira and H. Uchiyama: Determination of fish freshness using the K value and comments on some other biochemical changes in relation to freshness. *Sea Food Quality Determination* (D. E. Kramer and J. Liston ed.), 1987, Elsevier, Amsterdam pp. 185-220.
- 3) A. Cheyne: How the GM Torrymeter aids quality control in the fishing industry. *Fish. News Int.*, 14, 71-74 (1975).
- 4) A. C. Jason and J. C. S. Richards: The development of an electronic fish freshness meter. *J. Physics E. Scientific Instruments*, 8, 826-830 (1975).
- 5) N. Nakajima, K. Ichikawa, M. Komada and E. Fujita: Food chemical studies on 5'-nucleotides. 2. On the 5'- ribonucleotides in fishes, shellfishes and meats. *J. Agric. Chem. Soc. Japan*, 35, 803-811 (1961).
- 6) J. M. Ryder: Determination of adenosine triphosphate and its breakdown products in fish muscle by high performance liquid chromatography. *J.*

Agric. Food Chem., 33, 678-680 (1985).

- 7) T. Satio, K. Arai and M. Marsuyoshi: A new method for estimating the freshness of fish, *Bull. Japan. Soc. Sci. Fish.*, 24, 749-750 (1959).
- 8) K. Kato: Nondestructive measurement of fruits quality by electrical impedance (Part 1) - Relation between impedance parameters and freshness - Kansai Branch Report of the Japanese Society of Agricultural Machinery, No. 61, 29-32 (1987).
- 9) M. Sakaguchi and A. Koike: Freshness assessment of fish fillets using the Torrymeter and K-value. *Quality Assurance in the Fish Industry* (H. H. Huss ed.), 1992, Elsevier, pp. 333-338.
- 10) M. Ando, H. Toyohara, Y. Shimizu and M. Sakaguchi: Postmortem tenderization of fish muscle proceeds independently of resolution of rigor mortis. *Nippon Suisan Gakkaishi*, 57, 1165-1169 (1991).

#### FIGURE LEGENDS

Figure 1 Changes in K value (KV) of skin-on fillets during ice storage

Figure 2 Changes in Torrymeter reading (TMR) measured on skin side of skin-on fillets during ice storage.

Figure 3 Changes in Torrymeter reading (TMR) measured on bone side of skin-on fillets during ice storage.

Figure 4 Relation between the change in KV and that in TMR during storage. Mean values of TMR measured on skin and bone sides were plotted against KV. O-O, measured on skin side; O-O, measured on bone side

Figure 5 Changes in vector impedance trajectory (Cole-Cole arc) of skin-on fillets subjected to the measurement using needle type electrodes during ice storage. A, measured on skin side; B, measured on bone side

Figure 6 Changes in vector impedance trajectory (Cole-Cole arc) of skin-on fillets subjected to the measurement using wet contact type electrodes, during ice storage. A, Measured on skin side; B, measured on bone side  
O-O, Yt1; , Yt2; , Yt3.

Figure 7 Changes in radius of Cole-Cole arc of skin-on fillets measured on bone side. A, measured with needle type electrode; B, measured with wet contact type electrode Symbols are the same as given in the footnote of Figure 5.

Figure 8 Changes in difference of series equivalent resistance between 10K and 1MHz for skin-on fillets measured on boneside. A, measured with needle type electrode; B, measured with wet contact type electrode  $RS_{10KHz} - RS_{1MHz}$  (K ), difference of series equivalent resistance between 10K and 1MHz. Symbols are the same as given in the footnote of Figure 5.

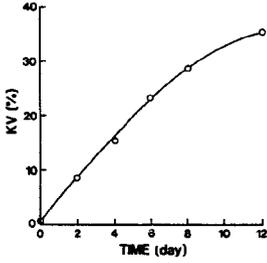


Figure 1

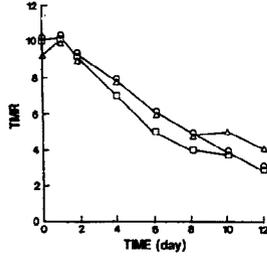


Figure 2

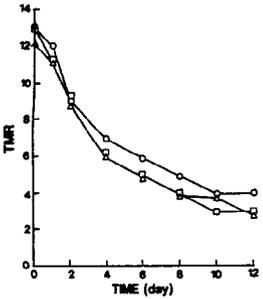


Figure 3

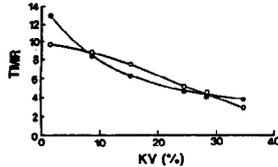


Figure 4

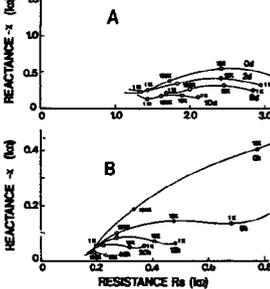


Figure 5

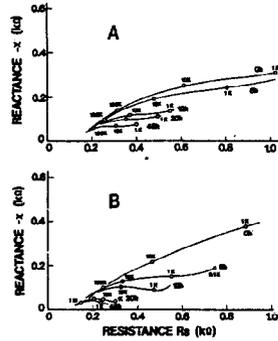


Figure 6

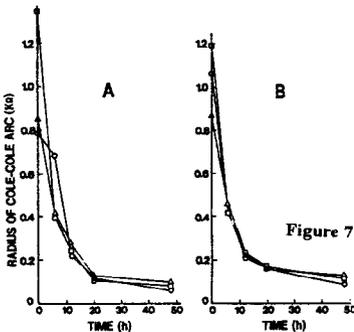


Figure 7

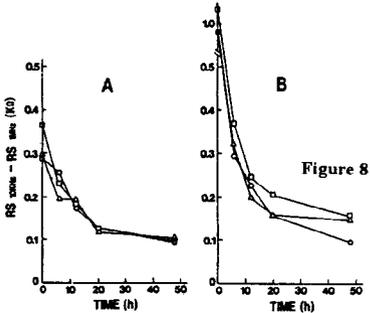


Figure 8

# PUBLIC HEALTH ASPECTS OF FOOD IRRADIATION\*

*Gerald Moy, Ph.D.  
Scientist, Food Safety Unit  
World Health Organization  
Geneva, Switzerland*

## SUMMARY

In view of the enormous health and economic consequences of foodborne diseases, the World Health Organization (WHO) encourages its Member States to consider all measures to eliminate or reduce foodborne pathogens in food and improve their supplies of safe and nutritious food. With the wholesomeness of irradiated food clearly established by extensive scientific studies, food irradiation has important roles to play in both ensuring food safety and reducing food losses. Food irradiation may be one of the most significant contributions to *public health to be made by food science and technology since the introduction of pasteurization*. Because the promotion of a safe, nutritious and adequate food supply is an essential component of its primary health care strategy, WHO is concerned that the unwarranted rejection of this process may endanger public health and deprive consumers of the choice of foods processed for safety.

## INTRODUCTION

From the earliest civilizations, all societies have had to develop means for ensuring adequate supplies of safe and nutritious food to meet the needs of their people. Ideally, such supplies would be of high quality, contain a wide variety of food items and be affordable to even the most disadvantaged members of society. In meeting these needs, humanity has had to confront a number of constraints including unpredictable variations in agro-climatic conditions, insufficient technical knowledge and inputs, inadequate infrastructure, seasonality of production and the inherent perishability of most food products. While various strategies for addressing these difficulties have been devised, it may be said that adequate food supplies could not have been achieved without some reliance on food processing and preservation technologies. This is particularly true of the contemporary world where a growing proportion of the population lives in urban centres far removed from the points of primary food production.

Current technologies include a variety of processes, some of which, such as drying, fermenting, pickling and salting, are of considerable antiquity, while others, such as fumigation, canning and pasteurization, are of more recent origin (see Figure 1). After more than forty years in research and development, treatment by ionizing radiation is beginning to be more widely used to supplement

\* Based on an article by Dr. Moy and F.K. Kaferstein which appeared in the Summer 1993 issue of the *Journal of Public Health Policy*

existing technologies for certain applications. One such application, which has significant public health potential, is the reduction of pathogenic microorganisms in solid foods. Food irradiation can also contribute to improvements in both the quality and quantity of the food supply, which means better health and nutritional status. Irradiation, as a process used to meet quarantine requirements, also holds great promise as an alternative to chemical fumigation and other methods for disinfestation. However, before considering these public health benefits, the scientific evaluations concerning the wholesomeness of irradiated food should be reviewed. After all, consumers have a right to expect that the highest standards of safety are met before food irradiation is permitted as a processing method.

### **Wholesomeness of Irradiated Food**

Before introducing any new food processing technology, adequate and reliable evidence must be evaluated to provide sufficient assurance that not only would the process produce the desired results in food but also that it would not have any unacceptable toxicological, nutritional or microbiological effects. For food irradiation, the gathering of this evidence at the international level was coordinated by the International Project in the Field of Food Irradiation, beginning in 1970. The adequacy and reliability of data generated by this project and from other sources were reviewed at a series of international meetings organized by the World Health Organization (WHO), often in collaboration with the Food and Agriculture Organization (FAO), and the International Atomic Energy Agency (IAEA). This series of international deliberations culminated in 1980 with the convening of the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food at WHO Headquarters in Geneva. In their report, this Committee concluded that the "... irradiation of any commodity up to an overall average dose of 10 kGy presents no toxicological hazard: hence, toxicological testing of foods so treated was no longer required" (WHO, 1981). It also found that irradiation up to 10 kGy "introduces no special nutritional or microbiological problems" These conclusions, then, clearly established the wholesomeness of any food irradiated up to a maximum absorbed dose of 10 kGy.

In subsequent years, a number of national and regional authorities convened their own expert committees to review and evaluate the data independently of the international review and evaluation conducted by WHO in collaboration with FAO and IAEA. Reviews were conducted, for example, in Denmark, France, the United Kingdom, the United States, and by the Scientific Committee for Food of the Commission of European Communities. No new concerns were raised by any of these reviews.

Based on these reviews as well as on information available at the time from other sources, the Joint FAO/WHO Codex Alimentarius Commission, in consultation with its Member Countries, reviewed the use of irradiation as a food technology and at its 15th session in 1983 adopted the Codex General Standard for Irradiated Food and the Recommended International Code of Practice for the

Operation of Radiation Facilities for the Treatment of Food. With the endorsement of Codex, the organizations involved hoped that countries would begin in earnest to apply food irradiation for the full benefit of the people, regardless of country's stage of development.

### **Updated Review of the Safety and Nutritional Adequacy of Irradiated Food**

Although there is nearly unanimous agreement within the scientific and regulatory communities regarding the safety and nutritional adequacy of food irradiated under the conditions defined, those opposed to food irradiation have continued to raise what they believe to be important and unresolved issues. By exploiting the public's fear of anything "nuclear", opponents of food irradiation have been successful in delaying the enactment of legislation to permit or expand its use in a number of countries. At the request of one such country, WHO in May 1992 convened a consultation to prepare an updated report on food irradiation based on a review of all relevant safety studies carried out since the 1980 Expert Committee, as well as on many of the older studies that had been previously considered. Such controversial issues as the - discounted - finding that irradiated food induced polyploidy in malnourished children, and the unsubstantiated claim that irradiation destroyed the nutritional value of food, were given particular consideration. After reviewing all the evidence, including over 200 toxicological studies, the group reaffirmed the earlier findings and concluded that irradiated food under established Good Manufacturing Practices (GMP) can be considered safe and nutritionally adequate (WHO, in press).

### **Public Health Aspects**

At first glance, the involvement of an international health organization such as WHO in the promotion of a technological process might be surprising. However, it is quite understandable once it is recognized that food irradiation has two applications that can contribute to the health and well-being of humanity, namely:

- (i) the elimination or reduction certain foodborne pathogens, thus making food safer, and
- (ii) the preservation of food by the destruction of pests and retardation of deterioration, thus increasing the supply of high quality food.

The food irradiation process has, therefore, the potential to contribute to one of the essential components of primary health care defined by WHO and UNICEF in their Declaration of Alma-ata in 1978, namely, the promotion of a safe, adequate and nutritious food supply.

### Improving Food Safety

In 1983, a Joint FAO/WHO Expert Committee on Food Safety concluded that foodborne diseases, while not well-recognized, are nevertheless one of the most widespread threats to human health and an important cause of reduced economic productivity (WHO, 1984). Foodborne diseases are caused by infectious or toxic agents that enter the body through the ingestion of food. Foodborne diseases are particularly serious for infants and young children, pregnant women, the immuno-compromised, the hospitalized and the elderly. Many of the estimated 7,000 people that die each year in the United States from foodborne diseases are in one of these groups (Roberts, 1989). While not suffering the high mortality rates as among vulnerable groups, the general population also suffers a very heavy burden from foodborne diseases, mainly salmonellosis, campylobacteriosis and shigellosis. Other foodborne diseases are caused by *Escherichia coli* (O157:H7), *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Clostridium perfringens*, as well as other foodborne microorganisms, such as protozoa, helminths and viruses. In the United States, the total annual incidence of foodborne diseases has been estimated at up to 99 million cases with direct and indirect costs of \$23 billion (US) (Garthright *et al*, 1988), while the reported cases of known bacterial causes of 6.3 million is only a small fraction of the extrapolated reality (Bennett *et al*, 1987).

Several other industrialized countries have similarly reported high incidences of foodborne diseases, particularly salmonellosis and campylobacteriosis (WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe, 1992). There is also strong evidence that this burden has been increasing in recent times. Figures 2 and 3 present the situations as reflected by official health statistics in England, Wales and Ireland (PHLS, 1989) and in Germany (SBW, 1991) respectively.

While epidemiological surveillance systems in developing countries are even less reliable, foodborne diseases in those countries are still commonly reported among the leading causes of both morbidity and mortality. The microbiological contamination of food is possibly responsible for up to 70% of the estimated one thousand four hundred million episodes of diarrhoea and 3.5 million associated deaths occurring annually among children under the age of five worldwide, mainly in developing countries (Esrey, 1990).

At least in industrialized countries, this dramatic increase in foodborne diseases appears to be related to increased consumption of foods of animal origin and, perhaps, to socio-cultural changes which have altered domestic food handling practices. While the framework of this paper does not allow us to examine in detail the various factors responsible for these rather disturbing statistics, two points should be made:

(i) The data reflected in the official health reports are but a small portion of the true incidence. WHO has reason to believe that the actual morbidity due to

foodborne diseases in many countries may be 100 or more times greater than that reported.

(ii) In countries with reasonably good epidemiological systems, animal-derived food products and especially poultry products have been identified as an important sources of disease, particularly salmonellosis and campylobacteriosis.

While health problems related to food contamination often differ between countries and regions of the world, the basic principles for prevention and control are similar. For this purpose, three lines of defense are available. The first line of defense is to improve the hygienic quality of raw foodstuffs at the agricultural and fisheries level. By applying accepted codes of good agricultural and animal husbandry practices, including improvement in the environmental conditions under which animals are raised, the hygienic quality of raw food products can be improved. Both WHO and the Codex Alimentarius Commission are actively involved in efforts to promote safer food at the primary production level. However, it is unlikely that certain foods of animal origin will ever be entirely free of foodborne pathogens.

The second line of defense is the application of food processing technologies, as mentioned at the beginning of this paper, which can prevent or reduce the transmission of foodborne pathogens as well as extend shelf-life. For example, in spite of the steady improvement in the hygienic quality of milk over the past one hundred years in most industrialized countries, no responsible public health authority would recommend abandoning the pasteurization process, since it is still not possible to consistently produce safe raw milk. In the case of raw poultry products, improvements in production methods can only be expected to reduce such pathogens as *Salmonella* and *Campylobacter* but not eliminate the contamination of raw products (Yule *et al.*, 1988).

The third and last line of defense is most critical to protect the health of consumers, especially when the first two fail. This concerns the education of foodhandlers on the principles of safe food preparation. Professionals as well as those responsible for food preparation in the home need to be more aware of the hazards posed by certain foods likely to be contaminated and to take care to assure they are handled safely. Greater efforts should be made by government, industry and other organizations, such as consumer groups, in this regard. However, for foods which are consumed in their raw or processed states without further preparation, consumers must rely on the first two lines of defense for protection against foodborne health hazards.

Because many foodborne pathogens are sensitive to doses of irradiation in the range of 2 to 7 kGy, the introduction of irradiation for the treatment of animal-derived food products, particularly poultry products, is expected to yield significant public health benefits as had the pasteurization of milk.

### Improving the Food Supply

While the reduction of foodborne disease is of considerable public health importance, the role of food irradiation in promoting nutritional status should not be overlooked, as good nutritional status is often considered synonymous with good health. For example, it is known that good nutritional status can ward off infections and reduce the risk of certain noncommunicable diseases, including cancer. Because good nutrition requires that food be safe, available and affordable, the food preservation capabilities of irradiation can make a contribution to improving both the quality and quantity of the world's food supply.

Some of the current applications of food irradiation in industrialized countries have been directed at increasing the diversity and quality of the food supply through improved shelf-life. Greater availability and affordability means that more consumers will have the opportunity to purchase a wider variety of foods. While this diversity has obvious nutritional benefits, consumers throughout the world appreciate some variety in their diets. Food irradiation can, therefore, make life healthier and, at the same time, more enjoyable, particularly in parts of the world where the food transport infrastructure is underdeveloped.

However, just assuring basic supplies of food may prove to increasingly difficult in the future and, here again, food irradiation may make a contribution. In view of the agricultural over-production of many industrialized countries, it may be hard for some people to imagine food shortages and famine becoming widespread. However, the earth's population continues to increase at an alarming rate. At the turn of the last century, our planet was inhabited by about 1.5 billion people. Our current global population of around five billion is expected to exceed the six billion mark by the year 2,000 and further substantial increases are likely. Most of these increases, about ninety percent, will occur in developing nations. In the face of limited arable land, declining soil fertility and water resources and potentially disastrous environmental change, it remains to be seen if all these people can be fed, much less fed nutritiously. With these ominous signs on the horizon, prudence would dictate that we take all measures necessary to prepare for any shortfall in the world's food reserves (WHO, 1992).

In this context, any preventable losses of food should be clearly targeted. In those developing countries where a warm climate favours the growth of spoilage organisms and hastens the deterioration of stored food, current losses are often enormous. In such countries, the estimated storage loss of cereals, grains and legumes is at least 10%. With non-grain staples, vegetables and fruits, the loss due to microbial contamination and spoilage is believed to exceed 50%. With commodities such as dried fish, insect infestation is reported to result in a loss of 25% of the product with an additional 10% lost due to spoilage (WHO, 1988). While not all of these losses can be prevented by food irradiation, the technology does offer unique potential to destroy insect infestation and reduce spoilage in order to effectively increase the supply of certain foods and, thereby, contribute to overall food security.

Low dose treatment by irradiation can also be used to meet quarantine requirements, a use which is becoming increasingly important as a number of chemical fumigants have been banned or restricted for various reasons. With few feasible alternatives, food irradiation may be particularly important for developing countries which rely on their food exports to earn important foreign currency.

### Public Acceptance of Food Irradiation

In a number of countries, consumer groups are opposed to food irradiation, their opposition often based on a lack of understanding of its real risks and benefits. However, food irradiation is not the first instance where public health advice on a new food technology has not been immediately accepted. Pasteurization of milk is a good case in point. When it was introduced about 100 years ago in North America, Europe, and other parts of the world, many milk consumers, as well as some scientists, voiced objections based on perceived hygienic, nutritional economic concerns (Hall and Trout, 1968). Today, pasteurization of milk is almost universally accepted as an essential public health technology that enjoys the confidence and support of the consuming public. Perhaps in a case where the exception proves the rule, milk-borne salmonellosis was a particular health problem in Scotland during the period from 1970 to 1982 when more than 3500 people fell ill and 12 died. After the introduction of milk pasteurization in Scotland in 1983, milk-borne salmonellosis virtually disappeared and can now only be found among those in the farming community who continue to drink raw milk (Sharp, 1988).

Whereas pasteurization was introduced mainly to interrupt the transmission of bovine tuberculosis, brucellosis and other milk-borne diseases, the most important public health applications of food irradiation are to destroy or reduce the ubiquitous and largely unavoidable pathogens that contaminate raw foods, especially those of animal origin. A Task Force on the Use of Irradiation to Ensure Hygienic Quality of Food concluded in 1986 that, at that time and in the foreseeable future, production of certain raw foods such as poultry or pork can not be guaranteed to be free from such pathogenic microorganisms as *Salmonella* and *Campylobacter* and such parasites as *Toxoplasma gondii* and *Trichinella*. In view of the declining quality of coastal waters in many parts of the world, shellfish species may also be considered likely candidates for irradiation to assure their safety and availability for human consumption, especially in reference to *Vibrios* such as *Vibrio cholerae*. Therefore, this Task Force believes that where certain foods are important in the epidemiology of foodborne diseases, irradiation treatment must be seriously considered (WHO, 1987).

WHO has incorporated this recommendation into its Golden Rules for Safe Food Preparation (Figure 4). The first of these ten Golden Rules advises the consumer to purchase foods processed for safety and gives as an example the recommendation to buy pasteurized as opposed to raw milk and to select fresh or frozen chickens which were treated with irradiation. Since only one of the ten

Golden Rules refers to irradiation, it is obvious that this technology can't be expected to produce food safety miracles. For this and other reasons, WHO has stressed that food irradiation should not be seen as a panacea to all the various food safety and supply problems humanity is facing. On the other hand, food irradiation is a perfectly sound food processing technology which can offer the consumer food products which have an additional margin of safety. Seen from this perspective, the unwarranted rejection of food irradiation is not only contrary to the public health, but also inconsistent with the rights of consumers to protect themselves and their families by choosing foods processed for safety.

### REFERENCES:

- Bennett, J.V., Holmberg, S.D., Rogers, M.F. and Solomon, S.L. (1987) Infections and parasitic diseases. In: Closing the gap: the burden of unnecessary illness, Amler, R.W. and Dull, H.B., eds. New York: Oxford University Press 102-14.
- Esrey, S.A. (1900) Food contamination and diarrhoea. World Health, January-February, 19-20.
- Garthright, W.E., Archer, D.L., and Kvenberg, J.E. (1988) Estimates of incidence and cost of intestinal infectious diseases in the United States. Public Health Reports 103, 107-115.
- Hall, C.W. and Trout, M.G. (1968) Milk pasteurization. Avi Publishing Company, Westport, Connecticut.
- PHLS (Public Health Laboratory Service) (1989) Surveillance of gastrointestinal infections: 1980-1989. Communicable disease report 89/52, December.
- Pariser, E.R. (1987) Post-harvest food losses in developing countries. In: Food policy: integrating supply, distribution and consumption, Price Gittinger, J., Leslie, J., and Hoisington, C., ed., Johns Hopkins Press, Baltimore.
- Röberts, T. (1989) Human illness costs of foodborne bacteria. Am. J. Ag. Econ. 71:468-74.
- SBW (Statistisches Bundesamt Wiesbaden) (1991), Meldepflichtige Krankheiten.
- Sharp, J.C.M. (1988) Milk-borne salmonellosis: the cost-effectiveness of control. Health and Hygiene 9:46-50.

WHO (1981) Wholesomeness of irradiated food. Technical Report Series No. 659. WHO, Geneva.

WHO (1984) The role of food safety in health and development. Technical Report Series 705. WHO, Geneva.

WHO ((1987) The use of irradiation to ensure hygienic quality of food. International Consultative Group on Food Irradiation (ICGFI) Task Force Meeting, 14-18 July 1986, Vienna, Austria. Document WHO/EHE/FOS/87.2. WHO, Geneva.

WHO (1988) Food Irradiation - a technique for preserving and improving the safety of food. WHO, Geneva.

WHO (1992) Report of the Panel on Food and Agriculture. WHO Commission on Health and Environment. WHO, Geneva.

WHO (in press) A review of the safety and nutritional adequacy of irradiated food. WHO, Geneva.

WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe (1992) Fifth Report 1985-1989. Institute of Veterinary Medicine - Robert von Ostertag Institute (FAO/WHO Collaborating Centre for Research and Training in Food Hygiene and Zoonoses), Berlin.

Yule, B., et al. Prevention of poultry-borne salmonellosis by irradiation: costs and benefits in Scotland. Bulletin of the World Health Organization (66) S:753-758, 1988.

**Figure 1. Current technologies for processing food.****DRYING**

Radiant (solar) drying  
 Air drying  
 Vacuum drying

Spray drying  
 Freeze drying

**FERMENTATION**

Yoghurt  
 Soya sauce  
 Vinegar

Cheese  
 Wine and beer  
 Sauerkraut

**CHEMICAL TREATMENT**

Salting  
 Sugaring  
 Fumigating

Pickling  
 Smoking  
 Food additives

**THERMAL TREATMENT**

Cooking (many forms)  
 Pasteurizing  
 High-temperature short-time

Blanching  
 Aseptic filling  
 Retorting

**COLD TREATMENT**

Refrigerating

Freezing

**MODIFIED ATMOSPHERE****MICROWAVE RADIATION****IONIZING RADIATION**

Figure 2. Incidence of foodborne diseases in England, Wales and Ireland (1980-89).

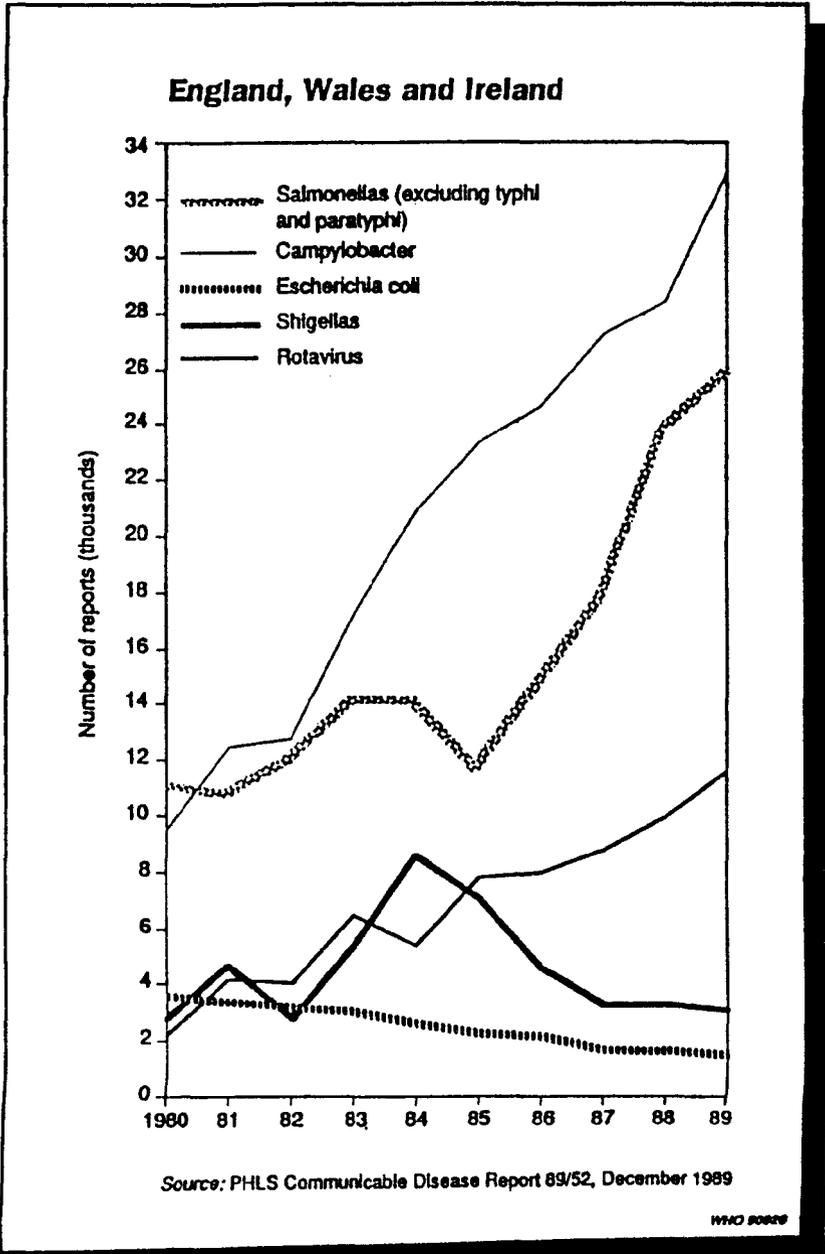


Figure 3. Infectious enteritis and typhoid and paratyphoid fevers in the geographical area of the Federal Republic of Germany (1946-89).

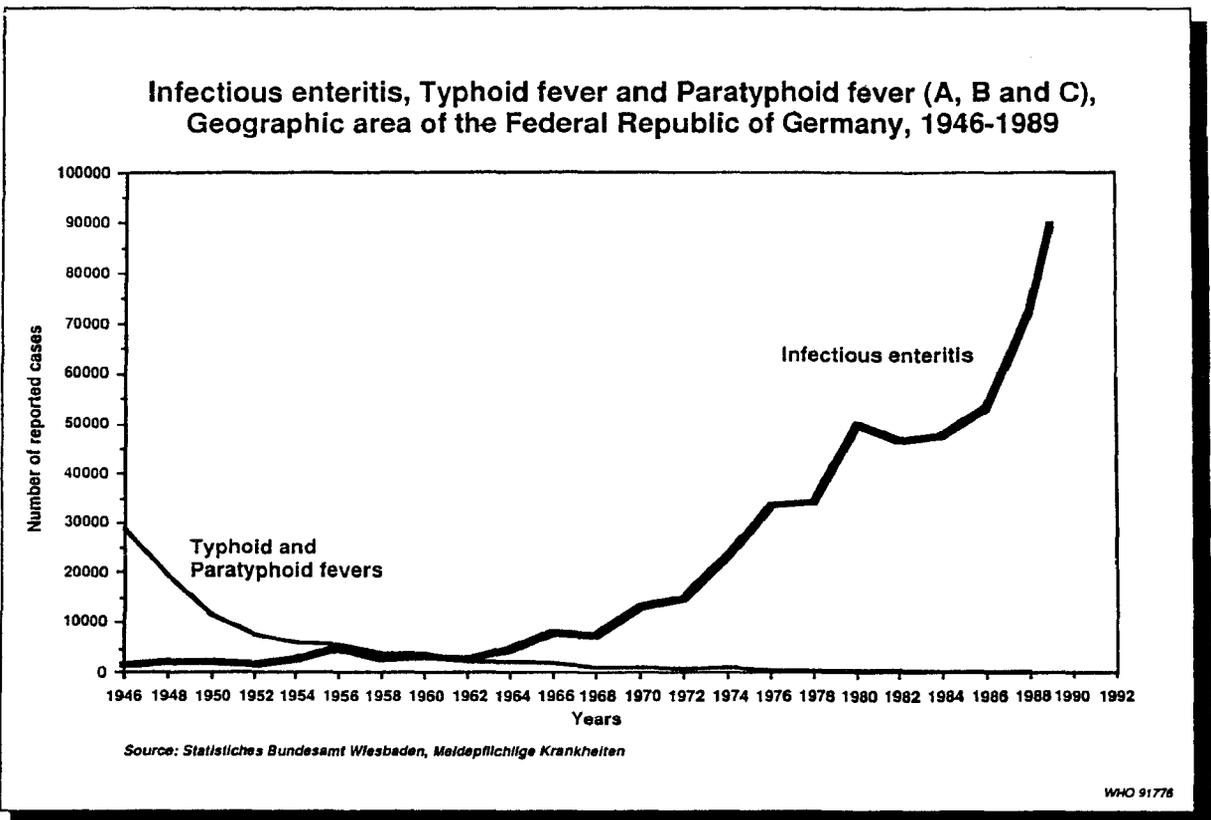


Figure 4. The WHO Golden Rules for Safe Food Preparation - First Rule

# The WHO Golden Rules for Safe Food Preparation

## **1. Choose foods processed for safety**

While many foods, such as fruits and vegetables, are best in their natural state, others simply are not safe unless they have been processed. For example, **always buy pasteurized as opposed to raw milk and, if you have the choice, select fresh or frozen poultry treated with ionizing radiation.** When shopping, keep in mind that food processing was invented to improve safety as well as to prolong shelf-life. Certain foods eaten raw, such as lettuce, need thorough washing.

WHO 99165



# DEVELOPMENT OF RAPID DETECTION METHODS FOR FOOD BORNE PATHOGENS IN HACCP PROGRAMS.

M.M. Garcia, B.W. Brooks, K.H. Nielsen, A.S. Denes and E.M. Pietrzak  
Animal Diseases Research Institute, Agriculture and Agri-Food Canada  
P.O. Box 11200, Station H, Nepean, Ontario, Canada K2H 8P9

## ABSTRACT

Recent well-publicized foodborne outbreaks have accelerated the establishment of the Hazards Analysis Critical Control Point (HACCP) programs as an integral part of many food processing systems of which microbiological surveillance of critical control points is an essential part. In the case of foodborne pathogens, a zero-tolerance in critical raw materials and finished products require that the microbiological tests be sensitive with high specificity. The monitoring of preharvest materials (animals) necessitates the development of more portable test kits to give on-site results. This presentation will include a brief description of some test on the market that are currently used in HACCP programs, and those being developed at ADRI. For the past 10 years, we have been involved in the development of rapid tests in support of the needs of the veterinary diagnostic and food safety programmes of Agriculture and Agri-Food Canada. We have focused our developmental efforts on a number of monoclonal antibody and nucleic acid-based assays for the rapid detection of *Salmonella*, *Campylobacter*, *Yersinia* and *Brucella*. These include various ELISA formats and the application of immunomagnetic beads for both trapping and detection. DNA-based assays using PCR amplification and RNA enhancement of PCR products have increased the level of detection sensitivity for these pathogens. Bioluminescence and chemiluminescence techniques also have been developed for estimating, very rapidly, total bacterial load and for predicting microbial quality and shelf-life of foods. Local and international collaborations with university, industry and other agencies on the transfer and field evaluation of these technologies are underway.

## INTRODUCTION

An estimated 80 million cases of foodborne illnesses and over 10,000 deaths occur yearly in North America (Todd, 1989). Most foodborne disease is caused by contamination of foods of animal origin, including shellfish. According to the Centers for Disease Control and Prevention in the U.S., *Salmonella* causes nearly two million cases and two to three thousand fatalities each year. *S. enteritidis* is a major concern because it can contaminate eggs by the transovarian route. *Campylobacter* accounts for more than two million cases and two to four hundred deaths. *Campylobacter enteritis* is the most

common diarrheal disease in most developed countries, including Canada. Improperly handled or undercooked poultry contribute to the high incidence of enteric campylobacteriosis. Verotoxic *E. coli* (VTEC), the culprit of the recent "Jack in the Box episodes" and popularly known as "hamburger disease" causes illness to ten to twenty thousand people in Canada with approximately three hundred deaths annually. *Listeria monocytogenes*, a bacterium commonly found in raw agricultural products causes illness to an estimated twenty-five hundred people with a relatively high mortality rate (25 to 35 per cent). While *Vibrio cholera* is causing serious losses in terms of lives and economies in many parts of the world, emerging pathogen, *Vibriovulnificus* has been found in U.S. coastal waters contaminating shellfish. About two dozen cases with a 50 to 60 percent death rate has been reported. These organisms are just a few of the increasing list of diarrheal pathogens that kill over 3.2 million people around the world every year.

Why the recent public concern for food safety? With the increasing frequency of family outings to fast-food establishments and an expanding variety of processed foods gracing the dining table, the risk of eating unsafe food becomes greater. For instance, a hamburger may come from an animal that was raised on one of over a million ranches in the U.S. and slaughtered in one of a thousand plants across the country. Or maybe, the meat came from a far-away country where it may have passed through many distribution channels before hitting the grill in any U.S. fast-food eatery. If food was mishandled along this circuitous chain, many could get sick with VTEC without the benefit of finding the source of the bacteria. The fresh or frozen broiler chicken one buys in the grocery store is likely to be contaminated with *Salmonella* and or *Campylobacter* in high enough numbers to cause illnesses when improperly cooked. About 500 *Campylobacter jejuni* cells are sufficient to cause diarrhea. A fresh chicken wing may contain about 10,000 *Campylobacter* cells. Cole slaw, potato salad, soft cheeses and liver pate containing *Listeria monocytogenes* can seriously affect the susceptible population such as pregnant women or immunocompromised persons.

### HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP)

The public's clamour for safer foods and the recent demand to upgrade the inspection system to deal with modern processing methods have led to a wider application of the HACCP principle. Originally developed by a team at the Pillsbury Company as a means of ensuring the safety of foods produced for the space program in the 70's, HACCP has re-emerged in the 90's as the primary monitoring system towards ensuring the safety of our food supply (Stier, 1993). This is reflected by the planned implementation of HACCP approaches in conjunction with inspection of meat and poultry and seafood by regulatory agencies in many developed countries. In turn, these agencies have encouraged food companies to seriously consider the concept. HACCP received a strong early support from the U.S. National Academy of Science report, which states that HACCP "provides a more specific and critical approach to the control of microbiological

hazards in foods than that provided by traditional inspection and quality control approaches.” (NAS, 1985).

In a HACCP-based operation, control of the safety of foods depends more on monitoring and controlling the physical and chemical features of the CCPs, such as the time/temperature regime used for pasteurization, than on determining the microbial content at any point in the process. Microbiological testing can show the possible existence of a hazard but (except in a few cases) does nothing to control it at that point. Also, it is fundamental to the HACCP approach that monitoring procedures provide warning of loss of control in time for corrective action to be taken. Conventional microbiological methods have limited applications in HACCP system because they are too slow and cumbersome. Techniques for monitoring must utilize a parameter that can be measured quickly and easily, so that action can be taken at the time a deviation occurs. Conventional methods are retrospective and do not provide any information about the behaviour of microorganisms in foods that may be important for assessing quality and predicting shelf-life of food products (Vasavada, 1993). Even today, few microbiological tests yield results in time to permit corrective action (Sharpe, 1994)

The terms of HACCP, defined by the International Commission on Microbiological Specifications for Foods (ICMSF 1988), recognizes two Critical Control Point levels. Thus, in some processes a single operation can completely eliminate one or more microbial hazards - this is a CCP1. Other steps which reduce but do not completely control a hazard - are called CCP2. The tendency today is to recognize only one level of CCP, and only to define CPs related to hazards (i.e. situations potentially able to result in food poisoning). This would avoid the problem associated with giving the impression that control of CCP1 is an absolute guarantee of safety when in reality, it simply means a risk with a higher level of assurance than that obtained by the control of CCP2. In 1989, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1989) proposed that a HACCP program include seven principles namely: assessment of risk, determination of critical control points, determining critical limits, monitoring the CCPs, corrective actions, record keeping, and verifying that the system works.

A number of areas in HACCP program that require microbiological testing are listed below (IAMFES, 1992; Stier, 1993; Sharpe, 1994.);

### 1. Risk Assessment and Identification of Critical Control

Points in a Process. Before initiating a HACCP approach, historical and published information including microbiological data about the product are needed to identify the critical control points. This may include generating in-house data of potential hazards. HACCP experts or consultants may be employed to assist in developing a HACCP program. Rapid methods allow the team to collect large amount of data more quickly and easily than traditional methods.

2. Critical raw materials for use in a product where no CCP1 is identifiable during processing. If the hazard is severe, a zero-tolerance criterion ( $c = 0$ ) will apply to such materials. The activity and purity of starter cultures should be checked before use. Development of on-site tests would benefit detection of pathogens at preharvest stage.

3. Monitoring of established CCPs ensure that there is no build-up of pathogens. Monitoring is the key to a successful HACCP program. However, even the most rapid of commercially available microbiological tests are not quick enough for on-line monitoring. Today it is known that many species of organisms rapidly develop biofilms (e.g. *Listeria*) that may provide protection from bactericidal agents at concentrations far above levels which kill the planktonic cells. Detecting the existence of biofilms, or their viability after cleaning and sanitizing, is becoming a major objective for rapid microbiological analysis.

4. Testing of critical finished products before their release to sensitive consumers. Again, the microbiological quality of the materials is a CCP1 and must be monitored before its use and should be considered under a zero-tolerance criterion.

#### 5. Verification of HACCP.

Microbiological testing also is necessary, to verify that a HACCP system is working. Verification is needed a) for a recently installed HACCP-based process; b) whenever a change is made in procedure, equipment, formulation, or practice, and c) as part of the continuous performance of an established HACCP program. Verification can be carried out internally or by independent parties such as auditors or regulatory agencies.

#### 6. Trouble shooting.

Unanticipated problems may occur that will require a quick resolution. Rapid tests are valuable in trouble shooting.

## CHARACTERISTICS OF RAPID TESTS

To be useful and acceptable, rapid methods for use in HACCP-based systems should have at least the following qualities (Stier and Blumental, 1993): a)

correlate with official and/or recognized methods, b) provide an objective index or marker, c) be relatively inexpensive for the value of data it provides, d) is safe to use in food production areas, e) correlate to food quality or safety, f) be field rugged and withstand the hostile environs of a farm or plant, and g) allow for remote evaluation or results.

## COMMERCIAL TESTS

In the present discussion of rapid tests, a review of some tests currently available in the market is warranted. There has been an explosion of technological advancements in this area and the time allotted for this presentation will only allow me to mention of a few that are widely used in food testing. Most of these are recognized by the Association of Analytical Chemists (AOAC approved or under consideration).

The first group of commercial tests involve those which are modifications of conventional plating or MPN techniques which generally are simpler and less laborious. Others combine both immunological or biochemical reactions associated with growth of the organism.

### Hydrophobic Grid Membrane Filter (HGMF)

This technique is inherently faster than the traditional plate count or MPN method for enumeration, but retains the need to grow the organisms to form countable colonies (Sharpe and Michaud, 194; Sharpe, 1989). Samples are filtered through the HGMF and the filter is then transferred to a special solid medium with specific indicator. Randomly distributed colony growth confined to square isogrids on the membrane are counted with an automated counter (HGMF interpreter) "Manual" readers (semi automated) are available. The HGMF has the capability of a wide counting range (105:1). Replicates (which are made easily and quickly from HGMFs) can be incubated on different media so that a biochemical profile can be obtained for the growths in each grid-cell or can be reacted with specific antibodies (Todd *et al.*, 1988). Interpretation of this is done by the HGMF Interpreter (Sharpe *et al.*, 1986). The HGMF was developed by the Canadian Health Protection Branch as a vehicle for automated counting. The Iso-Grid HGMFTM, the automated counter (HGMF Interpreter) and related system items are sold world-wide by two Canadian companies (QA Lifesciences Inc, 6645 Nancy Ridge Dr., San Diego, CA USA and Richard Brancker Research Ltd., 2 Monk St., Ottawa, Ont, Canada). In addition to techniques for total aerobes, several techniques for detection of pathogens have been developed.

### Petrifilm

Petrifilm<sup>TM</sup> represents a group of products designed to reduce manipula-

tions in bacterial enumeration. An advantage of Petrifilm (3M Company, St. Paul, MN, USA) is its simplicity and portability. No medium preparation is needed, and sample handling is simple and fast. Aseptic technique is required only while inoculating. The method is useful especially in laboratories where facilities for media production and incubator space are quite limited. The fluid food product or a dilution of the food is added to a dry culture medium coated on a 20-cm<sup>2</sup> film base. The bottom film containing growth medium is printed with a counting grid of 1 x 1 -cm squares and overlaid with a polyethylene film coated with water soluble gelling agent. To use petrifilm, the cover of the film is lifted and 1 ml of sample inoculated to the surface of the film. The film cover is replaced and a plastic spreader applied is placed on top of the inoculated area to ensure dispersal of the sample; the gel solidifies with 1 minute. Petrifilm plates are incubated at conventional temperatures and times. Petrifilm membranes are available for aerobic plate counts, coliforms, yeasts and molds.

### **Salmonella 1-2 Test**

Marketed by BioControl Systems Inc. (Bothwell, WA), the technique utilizes a combination of selective enrichment and immuno-diffusion to detect viable motile *Salmonella*. The method is conducted in a small disposable unit with two chambers. The smaller inoculation chamber contains a selective medium to allow the growth of *Salmonella* while inhibiting competitive bacteria. The larger motility chamber contains a peptone-based gel where a selective preparation of antibodies is added. If *Salmonella* is present, they will migrate during incubation through the selective medium towards the motility chamber and form a characteristic radial pattern with the antibodies which bind to the flagella.

### **Malthus instrument for conductance/impedence**

This is an automated system which is designed to detect growth patterns of bacteria by changes in electrical conductance. Microorganisms split large molecules to smaller mobile ions during their metabolism resulting in changes in the conductance (or impedence) of alternating electrical current. Within limit, the change in conductance can be related to microbial concentration. The Malthus instruments consist of high precision incubators, electronics control unit, a microcomputer running specific Malthus-AT software and a printer/plotter. Reusable sample cells contain a pair of platinum electrodes on a ceramic substrate. The cells may be inoculated through a septum and are connected to the incubators individually. When culture media are inoculated with organisms and monitored using conductance instruments, the plot of the change of conductance with time since inoculation, resembles that of a microbial growth curve (Gibson, 198). Conductance curves can be obtained at one-fifth to one-tenth the incubation times in conventional assays. Many "Total Count" measurements give a same-day result. Initial capital outlay, however, is quite high.

## ENZYME IMMUNOASSAYS

In the last two decades, enzyme immunoassays have become commonplace in clinical laboratories for disease diagnosis and for the rapid detection of a variety of compounds. Because it readily lends to automation, enzyme immunoassays are very useful in large scale screening and can be useful in HACCP systems. Various formats of enzyme-linked immunosorbent assay (ELISA) have been developed for the detection of foodborne pathogens. However, the limit of sensitivity of most ELISA techniques is about  $10^4$  to  $10^5$  cells per ml (Flowers *et al.*, 1986). This implies that an enrichment procedure should be done prior to detection ELISA, thereby reducing the "rapid" nature of the assay. Enzyme immunoassays can be broken down into two categories; homogenous and heterogenous assays. Homogenous assays require no physical separation of bound and free reagents because they work by monitoring the change in enzyme activity. They are convenient to perform but have limited sensitivity and are often affected by sample matrix. Heterogenous assays have separate steps including several washings between each step to remove unbound reagents. The enzymes most commonly employed include alkaline phosphatase, glucose oxidase and horse radish peroxidase. These enzymes catalyze reactions that cause colorless substrate to degrade and form colored products that can be observed visually or with a spectrophotometer. A number of tests, based on either manual (visual) or automated systems, have been on the market for a few years.

### Salmonella-Tek, Listeria-Tek

Developed by Organon Teknika (Durham, North Carolina, these tests are colorimetric monoclonal enzyme immunoassays which can be adapted for automation. The threshold value for the Salmonella-Tek is about 107-108 cells/ml (Sjerrod *et al.*, 1994). Because pre-enrichment and selective enrichment steps are employed, the assay takes a minimum of two days.

### Sinoclone Salmonella 1 & 2

A new kit released by Sinoclone Ltd. (Hong Kong) employs a two-step ELISA that detects *Salmonella* but also provides information of the serogroup. The latter is based on the well-characterized monoclonal antibodies against specific O-group LPS.

### Unique

Marketed by TECRA diagnostics (Roseville, NSW, Austria), the test employs an antibody-coated dipstick to first capture *Salmonella* present in a sample.

The *Salmonella* then is allowed to multiply in an enrichment M-broth to a level that can be detected on the dipstick by an enzyme immunoassay. A purple color indicates a presumptive positive reaction. Sample to result takes approximately 22 hours.

### Ridascreen Set Kit

The product which is produced by R-Biopharm GmbH, Darmsdt, Germany, utilizes monovalent capture antibodies against *Staphylococcus enterotoxin* types A to E. The kit has a high degree of specificity and sensitivity (0.20 to 0.30 ng of SEs per ml of certain food extracts and 0.5 to 0.5 per gram of foods such as noodles, ham, salami, cheese and turkey. It takes three hours to complete the analysis of individual enterotoxin types of SEA.

## NUCLEIC ACID PROBES AND AMPLIFICATION SYSTEMS.

Problems associated with bacterial classification and strain identification have been alleviated by the increasing applications of nucleic acid probes and monoclonal antibodies for the detection of foodborne pathogens (Terpstra et al. 1990; Thorne et al., 1990; Wetherall and Johnson, 1990; Wolcott, 1991). Thus the recognition that the ribosomal RNA (rRNA) sequence of a procaryote is a reliable basis for identifying the organism and for determining its evolution (Woese et al., 198) has gained wide attention. The inherent part of cells ubiquitous nature of ribosomes in all life forms is reflected in the high degree of conservation of rRNA sequences of related species interspersed with a mosaic of variable domains acquired through mutations in the course of the organism's evolution. There are 3 kinds of procaryotic rRNAs: 5 S, 16 S and 23 S containing approximately 120, 1540 and 2900 nucleotides, respectively. An interesting general approach for generating DNA probes based on amplifying variable regions of rRNA by polymerase chain reaction (PCR) was described by Barry et al (1990). They aligned limited sequences of eubacterial 16S RNAs and found two variable regions, designated V2 and V6, which exhibited greater variation between species than other variable regions. Since these were flanked by conserved sequences, primers for PCR based on these conserved sequences could be generated to amplify the variable tracts. The PCR-amplified regions could then be sequenced to create an appropriate probe for the organism. Specific probes can be designed even with only a 2 base different in the target sequences.

### Gene-Trak Systems

(Integrated Genetics, Framingham, Mass) has replaced earlier radioactive labelled probes with "dipstick"-type tests which make for relatively safe, simple

methods. The probes are not to DNA, but to specific fragments of 16S ribosomal RNA. While there is usually only one copy of a specific gene per cell there may be 1,000 - 10,000 copies of ribosomal RNA; testing for these instead gives a "leg up" on detectability. In the Gene-Trak dipstick kits, the probe has attached to it a "tail" of synthetic oligonucleotide, which carries a fluorescein label. First, the sample is enriched (1-2 days, depending on the organism). Target DNA exposed with NaOH is hybridized (in solution) to the probe. The dipstick itself is coated with a "capture" RnA complementary to the probe's synthetic tail. Then the dipstick, bearing captured probe, is immersed in a solution of HRP-labelled anti-flourescein antibody. Finally, the now enzyme-labelled dipstick is placed in substrate/chromogen mixture to yield a color reaction which can be read by a spectrophotometer.

### **Gen-Probe**

Unlike Gene-Trak which employs colorimetric methods, Gen-Probe utilizes chemiluminescence as the non-isotopic reporter system. Gen-Probe also user ribosomal RNA as the target molecule for the identification of microorganisms by DNA probe assays. The target molecule uses an acridinium tester attached to DNA probes which can be oxidized by hydrogen peroxide and hydrolyzed by a strong base to produce light, carbon dioxide and the oxidized acridone ring. The oxidative reaction is very rapid (one second) and is controlled automatically by the addition of detection reagents to the sample in Gen-Probes luminometer Leader 1 or 250 or the PAL.

### **Amplification systems**

The rapid development of target amplification systems which have revolutionized low level microbial detection in medical diagnosis will greatly benefit microbial detection in food safety. Foremost among these is PCR developed by Mullis and his colleagues at Cetus (1987): the *in vitro* specific primer-directed amplifications of DNA with a thermostable DNA polymerase. This assay can, in theory, amplify  $3.4 \times 10^7$ -fold. The size of the amplified region (target DNA) is generally 100-400 base pairs, although stretches of up to 2 kb can be sufficiently amplified (Saiki *et al.*, 1988). Naturally, all is not straightforward and there are many publications of procedures to cut down noise, spurious amplifications, sequence-scrambling, etc. Nevertheless, PCR (or equivalent reactions) promises to be very powerful if applicable to microbiol detection in foods. At least one practical problem is the huge mass of accompanying food material and other substances such as surfactants, from which one currently needs to extract organisms (or their DNA) in sufficient purity. These materials may be inhibitory to the PCR reaction (Rossen *et al.*, 1992).

Other less known amplification systems may eventually serve as attractive alternatives to PCR. These include Q-beta replicase, ligase chain reaction (LCR)

and nucleic acid sequence based amplification (NASBA). The Q-beta replicase system utilizes a new recombinant plasmid which carries the copy DNA sequence of MDV-1 (midi variant of phage QB RNA) attached to a T7 RNA polymerase promoter (Lizardi et al., 1988). The RNA molecules serve as both specific probe and amplifiable reported. The RNA-directed RNA polymerase, QB replicase, has the ability to synthesize large numbers of copies (a billion-fold amplification) from a small amount of template strand in half an hour. Unlike PCR, which uses DNA polymerase to create new pieces of DNA, LCR joins pre-formed stretches of DNA via a ligase enzyme. One these probes anneal with their complementary target sequences, the ligase can then join them. Like PCR, millions of copies of the original target can be produced in 20-30 reaction cycles. NASBA, developed by Cingene (Ontario, Canada) is an isothermal amplification system; all reactions take place at 42°C. It can also amplify either DNA or RNA sequences by a factor of 10 million in less than 3 h (Van Brunt, 1990). Provided that their costs will not be prohibitive, gene probes and their attendant amplification systems will find much use in the detection of major foodborne pathogens.

## BIOSENSORS

Biosensors technology is fairly young with the first paper appearing just 30 year ago. However, it may one day provide the food industry the monitoring technology it need to support an effective HACCP program. A biosensor is a specific biological sensing element in close proximity or linked (integrated) with a transducer that can sense the changes occurring as the biological elements reacts with a target (Goldschmidt, 1993). A biosensor employs the unique specificity of the biological molecule (sensors) for their target species (analytes). The sensors can be antibodies, enzymes, organelles, nucleotides, whole organisms, tissue cultures or tissues that may constitute biological recognition systems. This element may be protected by a membrane which is permeable to certain molecules in the environment. In addition to its ability to detect specific compounds, the biosensor must transmit an energy impulse proportional to the reaction of the biocatalyst with the analyte. The more concentrated the analyte, the more intense the signal change recorded by the transducer. The transducing part of the biosensor can be based on electrochemical, thermal, or optical instrumentation. Potentiometric systems measure changes in potential during a constant current. In many cases, enzymes or antibodies can be attached directly to pH electrodes and can even be bound to magnetically susceptible particles. The solenoid electrode is the "magnet". Antigen-antibody reactions can also be measured in potentiometric systems and could be used for detection of food and food-related pathogens (Ghindilis et al., 1992) In amperometric systems, reactions are measured as current changes that occur at a constant voltage. Salmonellae in foods have been identified by enzyme-linked amperometric immunosensors (Brooks et al., 1992). Microbial sensors using a lysogenic strain of *E. coli* immobilized onto an oxygen sensing electrode has been used to screen mutagens such as aflatoxins (Karube et al., 1989) since phage induction by mutagens de-

creases respiration of the lysogenic strain. Piezoelectric system is another electrochemical sensor which utilizes piezoelectric materials such as quartz or sapphire crystal. An electric field across a piezoelectric material induces a strain when an electrical field is alternated rapidly in polarity. This excites the material to oscillate or resonate with a measurable frequency (eg, 1- 10 MHertz) which depends on the total mass of the crystal including the mass coupled to the surface. As the mass increases during the reaction of sensor with analyte, the resonance frequency decreases. Antibodies to *Salmonella typhimurium* coupled to the crystal detected this organism because the frequency decrease with the added mass resulted from the antibody-antigen coupling (Luong, et al., 1990). Optical biosensors including fluorescence and luminescence will be discussed elsewhere in this article. Antibody coated sensors sometimes referred to as immunosensors for pathogen detection are also gaining much attention from rapid test developers.

Despite the potential of biosensors for on-site and on-line monitoring problems such as inherent instability of the biological sensing molecules and the short shelf-life of the instruments need to be resolved. In the future, an array of biosensors may be able to rapidly perform, with high reproducibility, specificity, and sensitivity, a variety of analytical procedures which will be valuable in HACCP programmes.

## **CHEMILUMINESCENCE AND BIOLUMINESCENCE-BASED METHODS**

Light emission is one of the relaxation pathways by which excited state molecules dissipate excess energy to stabilize to their ground states. Luminescence-based detection systems derived from chemical reactions (chemiluminescence) or those occurring in biological systems (bioluminescence) are extremely sensitive especially when integrated to efficient photon counting devices. The sensitivity range for chemi- or bioluminescent assay of any given analyte is usually higher ( $10^9 - 10^{15}$  M) as compared to fluorometry ( $10^{-10}$  M) and other spectrophotometric ( $10^{-7}$  M) methods. They are also excellent alternatives to radioactive labels because of their low cost, safety and ease of disposal.

Two types of bioluminescence-based assays are currently in place; (1) the luciferin-luciferase assay for ATP (McElroy and DeLuca, 1993) and (2) methodologies based on lux-gene technology or bacterial bioluminescence (Meighen, 1988). A more recent development using luminol chemiluminescence has been successfully demonstrated (Pietrzak et al., 1995) in the assay of microbial biomass with other advantageous features for on-site testing and will be discussed under our research program.

### **ATP Bioluminescence**

The basis for using ATP to estimate biomass is the ubiquitous distribution of ATP in all living cells, the rapid release from dead cells and the fairly uniform con-

centration of ATP in the protoplasm of all microbial cells. The ATP method is probably the fastest available indicator of total count (Wood and Gibbs, 1982; Bautista et al., 1993). Theoretical limits of detection are around 10-200 cells/g, however, practical limits may be 105 cells/g or greater. The success of the ATP determination for estimating bacterial numbers depends strongly on the efficiency of sample processing to separate bacteria from the food as well as inactivating somatic ATP before the assay.

The ATP bioluminescence assay can also be used to monitor hygienic conditions in processing plants. The very problem that makes the test of limited use in enumerating microbes in foods (high somatic count) can be used to advantage in monitoring surfaces in HACCP operations. Thus, although the ATP reading may not differentiate between microbes and food, high readings from surface swabs indicate that the cleanup was not complete. With a turnaround sampling time of approximately 10 minutes, a positive reading can be used to redirect the cleaning crew back to the area before processing is restarted (Kyriakades et al., 1990, Bautista et al., 1992, Seeger and Griffiths, 1994) Instrument and kits specifically for hygiene monitoring are now commercially available from various suppliers (Stanley, 1992.)

### **Genetically Engineered Luminescent Organisms**

While the use of ATP in a bioluminescent reaction, for detection of spoilage organisms, surface contamination and starter activity has not had significant application to the detection of pathogens, a new and exciting technology has recently been described. By recombinant DNA techniques, the lux gene from a naturally luminescent bacterium *Vibrio fischeri* is inserted in phages or bacteria (Meighen, 1988; Stewart 1990). The energy source is reduced flavin mononucleotide (FMNH<sub>2</sub>) rather than ATP. The specificity of host-phage interaction allows for the expression of the lux genes after successful lysogeny to the host which supplies the necessary substrates for the reaction. It has recently been demonstrated that lux genes inserted into phage P22 can be applied to *Salmonella typhimurium* and render it bioluminescent within 30 - 50 minutes (Stewart et al., 1989, Stewart, 1990). The limitation of this methodology is dictated in part by the intricate mechanisms involved in maintaining lysogeny over lysis in any given phage/host combination. Direct non-invasive detection of bacteria in any sample is possible without prior enrichment, subject only to the sensitivity of the photon counting device employed in the detection.

#### **Identification systems and test kits**

While no two systems correlate 100%, the reproducibility of commercial kits is probably better than the average laboratory achieves with conventional methods (Sharpe, 1994). Savings in analysis time, media preparation, and other labor are significant.

The proliferation of kits and instrumented systems in the last few years is staggering. All systems feature vials, strips, cards, dipsticks, etc, of prepackaged

media and reagents, often ingenious inoculators, and allow identifications based on detection of serological and chromosomal features, or biochemical or antibiotic susceptibility profiles. Some systems permit selection of tests. Many of the systems have paper detailing their performance, and various levels of technical support, including dial-in computerized diagnoses. A number of kits and systems have AOAC Official Action status. The growth of microorganisms in a clear broth medium increases its turbidity and absorbance. The change can be measured by a spectrophotometer.

### **bioMerieux Vitek AMS**

Several model (eg. Vitek Jr (30-test) to Vitek 60, 120, 240), vary in capability and price (bioMerieux Vitek Systems Inc., Hazelwood, MO, USA). Identification strips are available for Gram-negative non-Enterobacteriaceae (Rapid NFT), *Enterobacteriaceae* (Rapid E), *staphylococci* and *micrococci* (Staph Trac), *corynebacteria* (Rapid Coryne), *staphylococci* (RapiDec Staph), and *Salmonella*, *Yersinia enterocolitica* and *Shigella spp* (Rapid SYS). BioMerieux Vitek also sell the Bactometer impedance instrument as part of a complete system (Micro Team).

Practice cards are inoculated with a purified culture suspension are rotated so as to fill each biochemical test compartment. The card is placed in the instrument's incubator and is automatically read, optically, at regular intervals. Identification times from inoculation range from 2-48 h, depending on card, inoculum level, etc.

### **Microbial Identification System (MIS)**

Cellular fatty acid composition of bacterial cells is stable and a highly conserved genetic trait. Different bacteria possess different amounts and types of straight chain, hydroxy, branched and cyclopropane fatty acids of different molecular sizes. the Hewlett Packard MIDI 5898A includes HP Gas chromatograph, Computer, Automatic Injector, Sample Controller and Sample Tray). Fatty acids, extracted from pure cultures grown under specified conditions, are methylated to increase volatility and analyzed by the Hewlett-Packard MIS which is marked by Microbial ID. Fatty acid methyl ester (FAME) profiles are compared with a library of profiles for the organism or related species. This Gas liquid chromatography system is the only commercially available system of its kind for the rapid identification of microorganisms.

### **RAPID TEST DEVELOPMENT AT THE ANIMAL DISEASES RESEARCH INSTITUTE, NEPEAN.**

The Animal Diseases Research Institute is the largest laboratory in Canada devoted to the diagnosis and research of animal diseases. With the increasing eradication of major diseases such as Brucellosis and Bovine Tuberculosis, in-

stitute scientists in Microbiology and Immunology Sections were further given the task of developing rapid tests to support new food safety inspection programs. Thus far, our efforts have been directed mainly to *Salmonella* and *Campylobacter* since they cause the majority of foodborne illnesses and also are a major concern in the poultry industry. With the recent move towards global harmonization of trade, new food initiative programs such as the Pathogen Reduction Program of the USDA, and the Food Safety Enhancement Program of Agriculture and Agri-food Canada, have emphasized the need for rapid tests. This has gained support not only among regulatory clients and private agencies but also among technology industries. Our long-standing expertise in *Brucella* ELISA diagnosis has also provided the incentive to extend our monoclonal antibody work for rapid test development program.

Monoclonal antibody production and ELISA. An important consideration in our methods development program was to focus on producing high quality readends based on monoclonal antibodies (MAbs) and nucleic acid probes. At ADRI, we decided early on that our research should focus on the development of high quality reagents which can be applied to a number of rapid test formats whether developed in our laboratory or commercially available. In *Salmonella* testing, a number of monoclonal antibodies to epitopes of the core lipopolysaccharides of *Salmonella sp.* that do not cross-react with non-*Salmonella sp.* were produced (Tsang et al., 1991). A competitive enzyme immunoassay for detection of *Salmonella* was developed using one of these MAbs in order to provide a sensitive assay for same-day identification of *Salmonella* (Nielsen et al., 1993). The assay could be performed in about 90 minutes and consisted of the following steps. Lipopolysaccharide derived from *Salmonella typhimurium* was used to passively coat polystyrene 96-well plates and tubes. A titrated amount of monoclonal antibody specific for an outer lipopolysaccharide core epitope commonly found in *Salmonella sp.* was mixed with the prepared test sample prior to adding the mixture to the antigen coated matrix. Antibody bound to the immobilized antigen was detected with horseradish peroxidase labelled goat anti-mouse IgG (H&L chain). The analytical sensitivity of the assay was 3.1  $\mu$ g and 5.6 ng of lipopolysaccharide per ml for the plate and tube formats, respectively, if 25% inhibition or less was considered negative. This cutoff level was based on reactivity of unrelated monoclonal antibodies with *S. typhimurium* lipopolysaccharide and lipopolysaccharide from *E. coli* with the *Salmonella* specific monoclonal antibody in the assay.

Like other foodborne pathogens, the conventional method for detecting *campylobacters* has been the isolation of the bacteria by culture on laboratory media. In many cases, this is still the most reliable but also the most time-consuming and laborious method as many steps are involved in isolating and identifying the target organism from ubiquitous contaminants prevalent in foods and the environment. Recent successful development and commercial availability of rapid immunological and DNA-based assay for *Salmonella*, *E. coli* and *Listeria* have sharply reduced the turnaround times for testing these pathogens from approximately a week to a couple of days. Thus far, no reliable commercial ELISA test

has been developed for *Campylobacter* detection. Meanwhile, the demand for even more rapid (same-day or two hours) tests has dramatically increased in order to support newer and improved regulatory and industry food safety programs.

About five years ago, we characterized the antigens common to the 19 Lior serogroups most commonly isolated from humans, based on data obtained using both whole bacterial cells and sonic lysates of whole cells as antigens and produced MAbs against these antigens (Brooks *et al.*, 1992).

Evaluation by indirect ELISA indicated that several of the MAbs appear to be specific for the thermophilic campylobacters. One Mab, M357, reacted strongly with the *C. jejuni*, *C. coli* and *C. lari* reference strains. No cross-reactivity was observed with strains of *C. fetus* subsp. *fetus* and *C. sputorum* subsp. *bubulus* and 3 species of other bacteria, including *Citrobacter spp.*, *Salmonella spp.*, *Escherichia coli* and *Streptococcus faecalis*. M357 belongs to the immunoglobulin G1 subclass and reacted on Western blot with a 61 kDa component which has been identified as a flagellin protein. M35 could detect  $2 \times 10^5$  *C. jejuni* cells per ml in an indirect ELISA and is being evaluated in antigen capture or competitive immunoassays for the rapid detection of thermophilic campylobacters in food and clinical specimens.

To determine trapping efficiency, we developed a simple but powerful semi-quantitative of captured cells based on a Colony blot ELISA (Nielsen *et al.*, 1992). Direct observation as a complementary confirmation test has been done using both light and scanning electron microscopy

Immunomagnetic monoclonal based assays. A recent project we have initiated for on-site testing application is a new technology called immunomagnetic separations. This involves the use of antibody coated super paramagnetic beads for the rapid capture and detection of foodborne *Salmonella* and *Campylobacter*. The superparamagnetic beads consist of a magnetized core, covered in polystyrene either unaltered or with chemical groups attached for covalent linkages with biopolymers such as protein, lectins, antibodies and antigens (Ugelstad *et al.*, 1993). Basically, when magnetic beads coated with polyclonal or monoclonal antibodies specific for certain antigenic determinants (epitopes) of the target organism (foodborne pathogen) are applied in suspensions of environmental samples or emulsions of food containing the pathogen and contaminants, the antibody-coated magnetic beads can quickly "capture" the target organism. The captured organism can then be separated from the contaminated milieu by collecting the immunomagnetic beads with a magnet. This procedure is achieved in minutes. Despite the theoretical simplicity and elegance of this technique, there are many variables that can affect the efficient capture of the foodborne pathogens particularly where their original numbers are low. The impact of the immunomagnetic bead technology is quite apparent in the drastically reduced time of detection and, eventual, much lower cost per test because the only material required are the beads and an inexpensive magnet. The antibody coated beads can be easily stored in vials and transported to the field for on-site use or to laboratories for subsequent detection and/or monitoring purposes. Several studies have demonstrated the applicability of immunobead trapping for the rapid

detection of certain foodborne pathogens such as *Salmonella*, *E. Coli*, *Listeria* and *Yersinia* (Islam and Lindberg, 1992; Jackson et al., 1993; Luk and Lindberg, 1991; Skjerve and Olsvik, 1991) but scarcely on *Campylobacter*.

The adaptability of magnetic separation to several assays can offer many possibilities for increasing the efficiency of detection for foodborne *Salmonella* and *Campylobacter*. Advances in monoclonal based immunoassays, -flow cytometry, fluorescent and chemiluminescent assays developed at ADRI or elsewhere can also improve detection of immunobead captured antigens and microorganisms. For in-situ testing, the development of a one-step ELISA that can be read with an inexpensive hand-held spectrophotometer can be employed. In *Salmonella* detection, immunobeads could supplant the 24-h selective enrichment step in the conventional *Salmonella* detection method when followed by PCR or immunoassay (Fluit et al., 1993; Luk and Lindberg, 1991; Widjoatmodjo et al., 1992). We are improving biochemical and serological confirmation of viable *Salmonella* and *Campylobacter* by performing colony blot ELISA on differential medium plated with the immunobead captured target cells.

In any immunotrapping assay, the first major hurdle to be resolved is to determine what antibodies to exposed *Campylobacter* antigens can be used. The goal is to obtain maximal coating of magnetic beads with antibodies (IgG) that bind to many different surface epitopes of *Campylobacter* cells. We have used secondary antibody coated beads (anti-rabbit IgG) supplied by Dynal<sup>TM</sup> and coated them efficiently with primary MAbs antibodies (rabbit IgG). Coating efficiency can be titrated by ELISA. Optimization of conditions of trapping geared to the type of sample needs to be determined. Some of these factors include: size of beads, type of chemical group on the bead surface, type and titre of antibodies, biochemistry and cellular location of antigens of particular strains, binding affinity of antibody to captured target cells, cell to bead ratio, pH of medium, length and temperature of trapping conditions, use of enrichment media, etc. The overall goal is to attain a satisfactory procedure that can capture 1 to 100 cells from various pre-enriched or non-enriched samples with the shortest turnaround time possible (within 8-36h).

### **Immuno-magnetic separation with PCR (IMS-PCR)**

In addition to developing ELISA for detection, we are currently evaluating the use of PCR technology based on primers derived from DNA and/or rRNA sequenced probes developed in our laboratory or elsewhere. DNA primers based on published sequences of 16S and 23S RNA and gene probes, such as flagellar A and B genes, have been used for the rapid detection of thermophilic *Campylobacter*., *C. jejuni* and *C. coli* (Giesendorf et al., 1993; Oyofu et al., 1992). We are evaluating these primers on the basis of the spectrum of serotypes they are able to detect. Selected set of primers are being tested using actual field samples which may contain interfering substances. Fecal and other hemin-containing materials are particularly notorious for these. Once amplification is achieved, PCR products can be subjected to transcriptional enhancement

using a T7 phage RNA to increase sensitivity (Blais, 1994). Our studies have indicated enhancement of about a hundredfold with this combination compared to PCR alone. Published primers for *Campylobacter* PCR adapted in an assay format specifically using magnetic beads such as IMS-PCR or DIANA (Detection of Immobilized Amplified Nucleic Acid). Suitable primers will then be tried for either direct PCR and/or for DIANA (Detection of Immobilized Amplified Nucleic Acid) This method involves a set of nested primers and magnetic separation (MS) of the PCR-generated fragments (MS-PCR), followed by different signal transducing systems (Hornes *et al.*, 1991; Kapperud *et al.*, 1993). The nested PCR reduces the possibility of false results. The inner set of primers functions only if the outer pair has amplified the correct sequence, and therefore serves as a control of the first outer primer set. The inner primers are labelled, one with <sup>32</sup>P or qith a tail of partial sequence of the lac operator (lac Op) gene and the other with biotin. An alternative labelling method is the incorporation of digoxigenin-11-dUTP in the PCR reaction. The labelled amplicons are separated from the solution by streptavidin-coated magnetic beads in an Eppendorf tube or microtitre plate-format-magnets. The most common DNA sequence used for lacOp-labelled primer is a part of the lac operon gene: 5' AAT TGT TAT CCG CTC ACA ATT (Hornes *et al.*, 1991; Kapperud *et al.*, 1993; Wahlberg *et al.*, 1990).

### Rapid Chemiluminescent Technique

The principle of the luminol chemiluminescence for detecting bacteria is based on the activation of the oxidation of luminol monoanion by bacterial porphyrins in the presence of hydrogen peroxide (White and Roswell, 1972). The potential of this method for the detection and enumeration of bacterial populations in water samples has been explored before (Olaniacz 1970) and it is only recently that an adaptation of the methods was made applicable to food and environmental samples (Pietrzak *et al.*, 1995). Essentially by taking into account the factors affecting the kinetics of the reaction and the effect of potential interfering analytes, an optimum assay format was designed to span a considerable range of **analyte (heme) concentration from pure bacterial cultures**. The application of the assay in real life samples such as poultryrinses also gave good correlation between plate count and light output. Using a pre-sample processing step analogous to that used in ATP bioluminescence assay, preliminary results suggest a comparable degree of sensitivity. To substantiate this, more research is currently underway. The luminol assay appears to offer an alternative to the ATP assay in terms of cost (\$0.72 vs \$1000 per 1000 per 1000 samples), simplicity of use, suitability for automation and greater adaptability to field applications.

### Technology Transfer and Field Evaluation

With the assistance of the Industrial Relations office of Agriculture and Agri-Food Canada, we have been attracting other government labs, university and industry to participate at any stage project development for possible research

collaboration or licensign agreements. Thus far these agencies have shown a keen interest in the reagents and techniques we have developed. Their collaboration also is crucial to the successful transfer and field testing of the technology.

In conclusion, the increasing demands for safe and wholesome foods will accelerate the HACCP principles in regulatory agencies and industry. This will in turn, require more efforts in developing a new generation of improved rapid tests which can be installed for on-line or at-line monitoring in plants as well as for on-site testing for preharvest.

Finally, it must be mentioned that effective inspection program and monitoring programs should be supplemented with longterm public education and information programs. After all, food safety should be everyone's concern.

### REFERENCES:

- Bailey, J.S.,N.A. Cox and L.C. Blakenship. 1991. A comparison of an enzyme immunoassay, DNA hybridization, antibody immobilization, and conventional methods for recovery of naturally occurring salmonellae from processed broiler carcasses, *Journal Food Prot.*, 54 291-294.
- Barry, T., R. Powell and F. Gannon (1990). A general method to generate DNA probes fro microorganisms. *Biotechnology* 8 233-235.
- Bautista, D.A., L. McIntyre, L. Lalete and M.W. Griffiths. 1992. The application of ATP bioluminescence for the assessment of milk quality and factory hygiene. *Journal Rapid Methods and Automation in Microbiology*, 1 19-193.
- Blais, B.W., 1994. transcripitonal enhancement of the *Listeria monocytogenes* PCR and simple immunoenzymatic assay of the product using anti-RNA-DNA antibodies. *Applied and Environmental Microbiology* 60 348-352.
- Brooks, B.W., M.M. Garcia, R.H. Roberstson, D. Henning and H. Lior. 1993. Production and characterization of monoclonal antibodies to common antigens of *Campylobacter jejuni* and *Campylobacter coli*. *Acta Gastro-Enterologica Belgica*, Suppl. 1993:24.
- Brooks, J.L., B. Mirhabibollahi and R.G. Kroll. 1992. Experimental enzyme-linked amperrometric immunosensors for the detection of salmonellas in foods. *Journal of Applied Bacteriology* 73 189-196.
- Flowers, R.S., K. Ecker, D.A., Gabis, B.J. Robison, J.A. Mattingly and J.H. Siliker. 1986. Enzyme immunoassay for detection of *Salmonella* in foods:

- Collaborative study. *J. Off. Anal. Chem.* 69, 786-798.
- Fluit, A.C., M.N. Widjoatmodjo, A.T.A. Box R. Torensma and J. Verhoef. 1993. Rapid detection of salmonellae in poultry with magentic immuno-polymerase chain reaction assay. *Appl Environ Microbiol* 59 1342-1346.
- Ghindilis, A.L., O.V. Skorobogatko, V.P Gavrilova and A.I. Yaropolov. 1992. A new approach to the construction of potentiometric immunosensors. *Biosensors and Bioelectronics.* 301 - 304.
- Gibson., A.M., N. Bratchell and T.A. Roberts. 1988. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride, and storate temperature. *Int. J. Food Mircobiol.*, 6 155-178.
- Giesendorf, B.A.J., A. Van Belkum, A. Koeken, H. Stegeman, M.H.C. Henkens, J. Van Der Plas, H. Goosens, H.G.M. Niesters, W.G.U. Quint. 1993. Development of species-species DNA probes for *Campylobacter jejuni*, *Campylobacter coli* and *ampylobacter lari* by polymerase chain reaction fingerprinting. *J. Clinical Microbiology* 31 1541-1546.
- Goldschmidt M.C. 1993. Biosensors: blessing or bane? *Journal of rapid methods and automatic in Microbiology* 2 9-15. Hornes, E., Y. Wasteson and O. Olsvik, 1991. Detection of *Escherichia coli* heat stable enterotoxin genes in pig stool specimens by an immobilized, colorimetric nested polymerase chain reaction. *J Clin Microbiol* 29 23775-2379.
- IAMFES. 1991. Procedures to Implement the Hazard Analysis Critical Control Point System. International Association of Milk, Food and Environmental Sanitarians, Inc., Ames, IA., U.S.A.
- ICMSF. 1988. Microorganisms in Foods 4: Application of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality. International commission on Microbiological pecifications for Foods, Blackwell Scientific Publications, UK.
- Islam, D. and A.A. Linberg, 1992. Detection of *Shigella dysenteriae* type 1 and *Shigella flexneri* in feces by immunomagnetic isolation and polymerase chain reaction. *J Clin Microbiol* 30 2801-2806.
- Kapperud, G., T. Vardun, E. Skjerve, E. Hornes and T. Michaelson. 1993. Detection of pathogenic *Yersinia enterocolitica* in food and water by immunomagnetic separation, nested polymerase chain reaction, and color-

metric detection of amplified DNA. *Appl Environ Microbiol.*

- Karube, I., K. Sode M. SuZuki, and T. Nakahara, 1989. Microbial sensor for preliminary screening of mutagens utilizing a phage induction test. *Analytical chemistry* 61 2388-2391.
- Kyriakades et. al., 1990. Rapid hygiene monitoring using ATP bioluminescence. In *Bioluminescence and Chemiluminescence: Current Status*, eds P.E. Stanley and L.J. Kricka, John Wiley & Sons, Chechester, UK, pp 519-522.
- Lizardi, P.M., C.E. Guerra, H. Lomeli, I. Tussie-Luna and F.R. Kramer. 1988. Exponential amplification of recombinant-RNA hybridization probes. *Biotechnology* 6: 1197 -1202.
- Luk, J.M.C., and A.A. Lindberg, 1991. Rapid and sensitive detection of *Salmonella* (0:6,7) by immunomagnetic monoclonal antibody-based assays. *J. Immunol Meth* 13 1-8.
- Lund et al., 1988. Rapid isolation of K88-Escherichia coli by using immunomagnetic particles. *J. Clin Microbiol* 26 2572-2575.
- Luong, J.H., E. Prusak-Sochaczewski and G.G. Guilbault. 1990. Development of a new piezoimmunosensor for the detection of *Salmonella typhimurium*. *Annals of the New York Academy of Science* 613 439-443.
- McElroy, W.D. and M. DeLuca, In "Chemiluminescence and Bioluminescence" (M.J. Cormierr, D.M. Hurcules and J. Lee, eds.), p. 285, Plenum Press, New York, 1973.
- Meighen, E. 1988. Enzymes and genes from the lux operons of bioluminescent bacteria. *Ann. Rev. Microbiol.* 42. 151-176.
- Mullis, K.B. and F.A. Faloona (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155, 335-350.
- NACMCF, 1989.
- NAS, 1985. An evaluation of the role of microbiological criteria for foods and ingredients. National Academy of Sciences, National Research Council, National Academy Press, Washington, DC.
- Neilsen,, K.H., D. Gall, W. Kelly, D. Hening and M.M. Garcia. 1992. Enzyme Immunoassay. Application to diagnosis of bovine brucellosis. *Agriculture*

Canada, Ottawa.

- Nielsen, K.H., R.S.W. Tsang, M.M. Garcia, O. Surujballi and M. Nemeč. 1993. Competitive enzyme immunoassay for the detection of *Salmonella* lipopolysaccharide. *Journal of Rapid Methods and Automation in Microbiology* 1 305-314.
- Oyofe et al., 1992. Specific detection of *Campylobacter jejuni* and *Campylobacter coli* by using polymerase chain reaction. *J. Clin Microbiol* 30 2613-2619.
- Oleniacz, W.S. 1970. Chemiluminescent method for detecting microorganisms in water, *Environ Sci & Technol.* 2 (II), 1030-1033.
- Pietrzak, E.M., R.L. Kuhl and A.S. Denes. Estimation of total bacterial load by luminol chemiluminescence. In "Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects, (A. Campbell, L. Kricka and P. Stanley, Eds.) John Wiley & Sons Ltd., London 1995. In press.
- Rossen et al., 1992
- Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis and H.A. Erlich (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239 487-488.
- Seeger, K. and M.W. Griffiths 1994. Adenosine triphosphate bioluminescence for hygiene monitoring in health care institutions. *J Food Prot* 57 509-512.
- Sharpe, A.N. (1994). Rapid methods of microbiological analysis potentially applicable to HACCP-based operations. Unpublished.
- Sharpe, A.N. 1989. The hydrophobic grid membrane filter. In *Rapid Methods in Food Microbiology*, eds. M.R. Adams and C.F.A. Hope. Progress in Industrial Microbiology, Vol 26. Elsevier, pp 169 - 190.
- Sharpe, A.N. and G.L. Michaud. 1974. Hydrophobic grid-membrane filters: new approach to microbiological enumeration. *Appl. Microbiol.*, 28 223-225.
- Sharpe et al., 1986. Towards the truly automated counter. *Food Microbiol.*, 3 247-270.
- Sjerrod, P.S., G.A. June, W.H. Andrews, R.M. Amagnana and T.S. Hammack. (1994). Comparison of modified *Salmonella*-Tel enzyme immunoassay and modified Gene-Trak *Salmonella* assay for recovery of *Salmonella* from selected low- moisture foods.

- Skjerve, E., and O. Olsvik. 1991. Immunomagnetic separation of Salmonella from foods. *Int Journal of Food Microbiology* 14, 11-18.
- Stanley, P.E., 1992. A survey of more than 90 commercially available luminometers and imaging devices for low-light measurements of chemiluminescence and bioluminescence. *J. Bioluminescence and Chemiluminescence* 7, 77-108.
- Stewart, G.S.A.B., T. Smith and S. Denyer. 1989. Genetic engineering for bioluminescent bacteria. *Food Science and Technology Today*. 3, 19 - 22.
- Stewart, G.S.A.B. 1990. In vivo bioluminescence: new potentials for microbiology. *Letters Appl. Microbiol.*, 2, 17 - 26.
- Stier, R.F. 1993. Development and confirmation of CCP's using rapid microbiological tests. *J. Rapid Meth. Autom. Microbiol.*, 2, 17 - 26.
- Stier, R. F. and M.M. Blumental, 1992. The use of rapid methods for on-line monitoring. *Baking Snack* 14, 30-35.
- Terpstra, W.J., J.T. Schegget, and G.J. Schoone (1990). Detection of *Leptospira*, *Haemophilus* and *Campylobacter* using DNA probes. In *Gene probes for Bacteria*. New York, USA; Academic Press. pp. 295-322. [edited by Macario, A.J.L.]
- Thorne, E.M., A. Macone and D.A. Goldmann (1990). Enzymatically labelled nucleic acid (NA) probe assays for detection of *Campylobacter* spp. in human faecal specimens and in culture. *Molecular Cellular Probes* 4,133-142.
- Todd, E.C.D. 1989. Preliminary estimates of the costs of foodborne disease in Canada and costs to reduce Salmonella. *J Food Prot* 52,586-594.
- Todd, E.C.D., R.A. Szabo, P. Peterkin, A.N. Sharpe., L. Parrington, D. Bundle, M.A.J. Gidney and M.B. Perry. 1988. Rapid hydrophobic gride membrane filter-enzyme labels antibody procedure for identification and enumeration of *Escherichia coli* 0157 in foods. *Appl. Environ. Microbiol.*, 54, 2536-2540.
- Tsang, R.S.W., K.H. Nielsen, M.D. Henning, S. Schlecht and S. Aleksic. 1990. A murine monoclonal antibody specific for the outer core oligosaccharide of Salmonella lipopolysaccharide outer core. *Zentralblatt Bakteriologie*. 274, 446-455.

- Ugelstad *et al.*, 1993. Immunomagnetic separation of cells using magnetic polymer beads. In T.T. Ngo (Ed). *Molecular interactions in bioseparations*, Plenum Press, NY.
- Van Brunt 1990
- Vasavada, 1993. Rapid methods and automation in Food Microbiology: Beyond Delphi Forecast. *Journal of Rapid Methods and Automation in Microbiology*. 2, 1-8.
- Wahlberg, J., J. Lundberg, T. Hultman and M. Uhlen. 1990. General colorimetric method for DNA diagnostics allowing direct solid phase genomic sequencing of the positive samples. *Proceedings of the National Academy of Science USA* 87, 6569-6573.
- Wetherall, B.L. and A.M. Johnson (1990). Nucleic acid probes for *Campylobacter* species. In *Gene probes for Bacteria*. Orlando, Florida: Academic Press. 256-293. [edited by Marcario, A.J.L.]
- White, E.H. and D.F. Roswell. 1972. The chemiluminescence of organic hydrazides. *Res. Accounts* 3, 54- 62.
- Widjoaatmodjo *et al.*, 1992. The magnetic immunopolymerase chain reaction assay for direct detection for salmonella in fecal samples. *Journal of Clinical Microbiology* 30 3195- 3199.
- Woods and Gibbs (1992). New developments in the rapid estimation of microbial populations in foods. In: *Applied Science Publishers*, Ed.: R. Davies, Applied Science Publishers, London & New Jersey.
- Woese, C.R.E. Stackebrandt, T.J. Macke and G.E. Fox (1985). A phylogenetic definition of the major eubacterial taxa. *Systematic Applic Microbiology* 6, 143 - 151.
- Wolcott, M.J. 1991. DNA-based rapid methods for the detection of foodborne pathogens. *Journal of Food Protection*, 54, 387 - 401.



# ANALYTICAL TOOLS FOR THE EVALUATION OF FOOD QUALITY AND SAFETY

*Agnes C. Torres,  
Technical Consultant, MERCK, Inc.*

## ABSTRACT

This paper presents the use of the different methods in the evaluation of food quality and toxicity, the following methods, namely, the use of chemical and enzymatic test kits, high performance liquid chromatography and microbiological methods will be discussed.

The chemical test will be applied to the analysis of cations and anions in food matrices, like sausage and meat products. On the other hand, analysis for total dietary fibre, aspartame, ethanol, cholesterol, glucose/fructose/sucrose, lactose/lactulose can be analyzed by using enzymatic test kits called "BIOQUANT."

More complex food substances, for example, analysis of mycotoxins, preservatives, vitamins as well as, products of fermentation can be analyzed using high performance liquid chromatography.

A very important test for food products is microbiological analysis. The flowcharts for microbiological analysis of the following food products will be shown: MEAT, FISH, EGG, MILK, DRIED FOODS, ICE-CREAM, NON-ALCOHOLIC REFRESHING DRINKS (FRUIT JUICES), BEER AND CEREALS. Special emphasis will be given to the use of rambach agar for the 24-hour analysis of *Salmonella*.

Mobile analysis was developed as a supplementation of classical laboratory analysis. The possibility of on-the-spot analysis at the sampling site saves you the tedious transport of samples back to the laboratory and in addition, allows immediate measures to be taken without delay. At the same time you can count on low analytical requirements and a low cost per analysis.

Mobile analysis also finds application as a screening agent in the laboratory because with the prior determination of uncritical samples, unnecessary measurements with expensive analytical instruments can be avoided, saving time and money.

Three ready-to-use test systems (sometimes called quick tests) play a role in mobile analysis these are:

Aquamerck

The ready-to-use tests for easy and fast measurements, e.g., of surface waters.

### Aquaquant

The ready-to-use tests for the measurement of low concentrations, e.g., of contaminants in drinking water.

### Mircroquant

The robust ready-to-use tests for the analysis of even turbid solutions, e.g., waste water.

Further information on professional analyses using ready-to-use tests and their proper application are available more than 30 parameters from A for aluminium to Z for zinc can be determined with the ready-to-use test developed by Merck.

Detailed product information indicates areas of application (e.g., water recovery, swimming pools, construction industry) and gives details about measuring ranges, methods, and number of determinations so that you can just pick the ready-to-use test for your application.

## ENZYMATIC FOOD ANALYSIS

Presented here is the enzymatic method of food analysis written by Karin Bauer, Insitut Fresenius, Department of Food Analysis, Taunusstein, Federal Republic of Germany.

Enzymatic analysis constitutes an independent method in analytical chemistry. In the field of food analysis, it allows the determination of many food constituents, in the concentration range of major components as well as in the trace range. Enzymatic analysis are employed in both public and private analytical laboratories in industry and in research.

The influence of enzymes on fermentation processes, the saccharification of starch, or the presence of saccharase in honey was already known in the 17th and 18th centuries. Moreover, work on enzyme assays has been in progress for a long time in the field of food chemistry; for example, such enzymes are indicative of the food condition (freshness, pasteurization, sterilization). The measurement of enzyme activities is employed for this purpose. In 1935, Otto Warburg discovered the hydrogen-transferring pyridine coenzymes and the associated enzymes, and thus rendered possible the first steps toward routine analysis.

### Enzymes and Coenzymes

Enzymes are proteins which exhibit catalytic effects. A catalyst is defined as a substance which accelerates a thermodynamically possible reaction without itself being consumed in the reaction. Enzymes act in both intracellular and extracellular areas of the organism. They consist of about twenty different amino acids with a chain length between 100 and 2000 amino acid units, and have the

shape of a helices or leaflet structures, or mixtures of the two. Practically all metabolic processes are controlled by enzymes. For this purpose, the enzymes must possess a high specificity; that is, a given enzyme catalyzes the conversion of only one specific substance (substrate) to a definite final product. In addition, the enzyme causes a high conversion rate. To induce the chemical reaction, the substrate must fit into the enzyme in much the same way as a key fits into a lock (key-and-lock theory of E. Fischer). Enzymatic reactions are characterized by the fact that they proceed under physiological conditions, that is, at room temperature, at nearly neutral pH values, in aqueous solvents, and under atmospheric pressure. Necessary components for enzymatic reactions are compounds of low molecular mass, the coenzymes, which are more or less strongly bound to the enzyme.

Coenzymes frequently employed in the chemical analysis of foodstuffs include nicotinamide-adenine-dinucleotide (NAD) and phosphate (NADP). Their coenzyme function is reversible hydrogen acceptance or donation. During this reaction, a chinoid system is formed from the aromatic pyridinium system; the optical absorption in the near ultraviolet thereby shifts. The chinoid system exhibits an additional absorption maximum of 340 nm. Since the consumption or formation of NAD(P)H is usually proportional to the quantity of substrate to be determined, a quantitative analysis of the substrate can thus be performed. If the substrate under investigation exhibits an optical absorption similar to that of the coenzymes, a subsequent colour reaction permits an evaluation.

A prerequisite for the use of enzymes for the chemical analysis of foodstuffs is their isolation from animal and vegetable materials with the purity required for analytical purposes.

### **Sample preparation and measurement**

During recent years, the enzymatic analysis of foodstuffs has become established in routine analysis. The official collection of analytical methods in 35 of the Lebensmittel-und Bedarfsgegenstandegesetz (Act Relating to Foods and Commodities) in the Federal Republic of Germany already includes more than forty enzymatic methods; the situation is similar in other countries. By virtue of the substrate specificity, enzymatic techniques of analysis serve as reference methods.

The enzymatic analyses performed for substrate determination are final value methods; that is, the equilibrium of the enzymatic reaction is situated entirely on the product side, or is shifted to the product side by experimental measures.

Because of the high substrate specificity, no complicated sample preparation is required for enzymatic analysis. Dilution of the sample material with distilled water, for example, for the analysis of fruit juices, is often quite sufficient. If an intensely coloured sample must be employed, decolourizing can be accomplished with absorbents such as PVPP or polyamide; however a mixing test must

then be performed to ensure that no substrate is absorbed.

For turbid samples, filtration or centrifugation is frequently sufficient. Foods with a high protein content can be prepared by Carrez clarification or perchloric acid precipitation with subsequent filtration. For foods with a high fat content, removal of the fat by freezing at 4°C with subsequent pipetting or cold filtration is recommended.

Double distilled water or water of comparable purity should be employed for preparing enzyme solutions, coenzyme solutions. The pipetting volumes range between 0.05 and 2.0 ml. In the laboratory piston stroke pipettes with disposable plastic tips are usually employed. In the case of serial analysis, the measurement is performed in disposable plastic cuvettes. Two types of photometers are employed: Individual, fixed wavelengths are available for measurement with the line photometer, whereas the optical wavelength can be selected as desired with the spectrophotometer. In the latter case, linearity and correctness must be ensured for the indication withing the extinction range from 0 to 2.0 with + 1 per cent of the indication. The high extinction value of 2.0 is necessary, since for some reactions the procedure starts with a reduced coenzyme; consequently, high initial extinction values must also be correct (for example, in the determination of glycerol and citric acid). The calculation of the analytical results from the differences in extinction is based on the Lambert-Beer law.

For checking the reagents, especially the enzymes, the simultaneous analysis of a standard solution of known concentration is recommended. In the case of difficult sample materia, the sample preparation should be checked by means of a mixing test. The "Schweizer saccharose test" is suitable for use as a check on the working procedure, the photometers, and the accesories.

The advantage of substrate specificity offered by enzymatic food analysis in comparison with other chemical methods has already been mentioned. The sensitivity is about 80 mg/l for the example of coenzyme NADH measurement for the determinatin of saccharose. If the sample volume is increased from 0.1 to the attainable value of 2.0 ml, a sensitivity of 4 mg/l is obtained.

If the variational coefficients are compared, for example, for various methods of sugar determination, the values for chemical, physicochemical, and enzymatic analyses are of the order of 1 to 5 per cent. An experienced laboratory assistant can certainly achieve a variational coefficient of 1 percent for enzymatic analysis.

### Possible Errors

As far as the susceptibility of enzymatic analysis to error is concerned, the inhibitory action of heavy metals such as cadmium, lead, copper, mercury and silver must receive special mention. Side chains of the enzymes can form complexes with these metals and thus block enzymatic catalysis.

Enzymatic reactions proceed under physiological conditions. This implies, among other items, that a pH optimum exists for every enzymatic catalysis. By means of the buffers employed during the analysis, this pH optimum is adjusted

in the cuvette. Shifting of the pH value (due, for example, to faulty sample preparation), can result in interference with the equilibrium reaction, and thus with the entire analysis.

Interference with the reaction can also result from foreign activity caused by contamination of enzyme preparations. Activity of this kind gives rise to creep reactions; that is, the extinction being measured increases (or decreases, as the case may be) continuously, without reaching an end point. Extrapolation is possible in this case.

### **Automation of enzymatic analysis**

As dictated by the particular method, the time required for an enzymatic analysis ranges from ten minutes to one hour. More tedious sample preparation with certain materials, for example saponification for cholesterol determination, have thereby not been taken into account. With many methods, up to fifteen samples can be analyzed together in series.

Automation is already feasible for enzymatic analysis. In the field of clinical chemistry, where enzymatic analysis is now an indispensable technique, equipment is available for automatic analysis and is capable of performing hundreds of analyses per hour. The centrifugal analyzer is for example, employed for food analysis. The method is based on the principle of centrifugal mixing and subsequent measurement in rotating cuvettes. Pipetting of samples and reagents into a cuvette rotor is accomplished by microprocessor-controlled, articulated arms. The measurement is performed horizontally in the cuvette in the longitudinal direction of the beam path of the spectrophotometer with grating monochromator. For an enzymatic determination with an average duration of twelve minutes, up to 150 samples can be analyzed every hour, since different enzymatic analyses can also be conducted in parallel by means of appropriate programming. In the case of fruit juices, enzymatic analysis thus allows the determination of glucose, fructose, saccharose, sorbitol, malic acid, and citric acid as routine parameters.

A further possibility of automation is that of flow injection analysis, where the flow through principle is applied. The sample is introduced into a transport stream to which the reagents are added, and the measurement is subsequently performed photometrically in a flow through cuvette. In the case of the segmented flow system of analysis, the individual samples are mutually separated by air bubbles. This approach is applied, for example, in the analysis of wine for determining glucose and fructose.

### **Examples of enzymatic application**

Finally, the enzymatic determinations most frequently applied in food analysis are briefly described. The sugars, glucose, fructose, saccharose and lactose, thereby deserve special mention. Refreshment beverages such as lemonades, fruit juices, alcoholic beverages (beer, wine) as well as solid foods, for example,

chocolate and dietetic foods, are analyzed for these sugars. Formerly, only the unspecific reductometric Luff-Schoorl analysis was applied. On the one hand, this approach permits only a rough differentiation; on the other hand, the result of the analysis can always be affected by other reductones which are not sugars. In the case of carbohydrates, the enzymatic analysis of starch, for example, in bakery goods as well as in meat products (meat balls and patties) should be mentioned.

In the field of fruit juice analysis, the spectrum of organic acids is determined, in addition to the sugars, for checking the identity of a juice. The enzymatic analysis of citric acid, isocitric acid, and L-malic acid are worthy of mention here. Moreover, the L- and D-lactic acids produced during fermentation can be checked. A further advantage of enzymatic analysis in this connection is the fact that it is not only substrate-specific, but also stereospecific, as in the example of L- and D-malic acids. Attempts have already been made to determine D-malic acid besides L-malic acids, in order to detect a further possible adulteration of juices.

For laboratories with modest instrumentation, the enzymatic determination of the amino acid, asparaginic acid, allows the appraisal of fruit juices without the need of an expensive amino acid analyzer.

The enzymatic analysis of nitrates is also worthy of mention here. Nitrate reductase, which reduces nitrate to nitrite, can be substituted for the cadmium reductor, whose toxicity is objectionable.

The enzymatic determination of cholesterol presents an alternative to gas-chromatographic analysis, which requires expensive apparatus and personnel.

The methods mentioned represent only a cross-section of the enzymatic spectrum in the field of food analysis.

### Conclusions

By virtue of its high substrate specificity and high sensitivity, enzymatic analysis is well suited for the chemical analysis of foods. The sample preparation is usually simple and thus saves time during routine laboratory analysis. For a sufficient number of samples, automation is also feasible. The analytical methods presented as examples partially illustrate the existing spectrum of applications in the field of food analysis.

**REFERENCES:**

- Baltes, W., Schnellmethoden zur Beurteilung von Lebensmitteln, Behr's Verlag, Hamburg (1987)
- Belitz, H.D., Lehrbuch der Lebensmittelchemie, Springer-Verlog, Berlin (1982)
- Bergmeyer, H.U., Methoden der enzymatischen Analyse, Verlag Chemie, Weinheim (1977).
- Enzymatische Lebensmittelanalytik, Boehringer Mannheim GmbH (1978)
- Gambocz, E., Deutsche Lebensmittel-Rundschau 77, 1-15 (1981)
- Henniger, G. Flussiges Obst. 3, 111-127 (1984)
- Henniger, G. Zeitschrift fur Lebensmittel- Technologie und Verfahrenstechnik 30, 137-144, 182-185 (1979)
- Henniger, G. CLB Chemie fur Labor und Betrieb 39, 2/88, 5/88, 10/88, 1/89, 3/89, 10/89, 11/89Scheuermann, C., Confructa 1-, 162-176 (1986)
- Ugrinovits, M. Alimenta 19, 155-159 (1980)
- Ugrinovits, M. Chromatographia 13, 386-394 (1980)
- Walter, E. alimenta 19, 159 - 164 (1980)

**PRODUCT INFORMATION/ORDERING INFORMATION**

Procedural instructions for enzymatic foodstuffs analysis with single reagents.

Procedural instructions for analysing twelve parameters in foodstuffs with the help of reagents that have been tested in trials are offered by the collection of regulations "BIOQUANT-Enzymatic analysis in foodstuffs". This collection is updated continually.

**ENZYMATIC ANALYSIS IN FOOD USING BIOQUANT****SUBSTANCES THAT CAN BE DETERMINED USING BIOQUANT**

- ACETALDEHYDE
- AMMONIA (UREA/AMMONIA)
- CHOLESTEROL

- ASPARTAME
- ETHANOL
- CITRIC ACID (CITRATE)
- FRUCTOSE
- FORMIC ACID (FORMATE)
- (GLUCOSE/FRUCTOSE/SUCROSE)
- GLUCOSE
- L-GLUTAMIC ACID
- (GLUCOSE/SUCROSE)
- ISOCITRIC ACID (D-ISOCITRATE)
- GLYCEROL
- L-LACTIC ACID (L-LACTATE)
- D-LACTIC ACID (D-LACTATE)
- LACTULOSE
- LACTOSE (LACTOSE/LACTULOSE/  
GLUCOSE/FRUCTOSE)
- L-MALATE
- MALTOSE (GLUCOSE)
- NITRATE
- M-SORBITOL
- SUCROSE
- D-SORBITOL
- STARCH (GLUCOSE)
- TOTAL DIETARY FIBER
- UREA/AMMONIA
- TRIGLYCERIDES

**WHAT SAMPLES ARE AMENABLE TO HPLC ANALYSIS?**

SAMPLES MUST BE SOLUBLE IN A SOLVENT. IT IS THE METHOD OF  
CHOICE FOR THE ANALYSIS OF:

- NON-VOLATILE SUBSTANCES
- SUBSTANCES WITH HIGH POLARITY OR IONIC SAMPLES
- SUBSTANCES WITH HIGH MOLECULAR WEIGHT
- THERMALLY UNSTABLE AND DECOMPOSABLE  
SUBSTANCES

APPLICATION FIELDS INCLUDE:

- ORGANIC CHEMISTRY
- PHARMACEUTICAL CHEMISTRY
- CLINICAL CHEMISTRY
- FORENSIC CHEMISTRY
- FOOD CHEMISTRY

- COSMETICS
- BIOCHEMISTRY
- POLYMER CHEMISTRY
- ENVIRONMENTAL CHEMISTRY

TYPICAL ANALYSES INCLUDE:

- AFLATOXINS
- ALDEHYDES
- AMINES/AMINO ACIDS
- ANTIBIOTICS
- ANTIOXIDANTS/PRESERVATIVES
- CARBOXYLIC ACIDS
- DYESTUFFS
- FATTY ACIDS/ESTERS/LIPIDS
- WATER
- VITAMINS
- XANTHINES
- PURINES
- HERBICIDES/PESTICIDES
- HORMONES/STEROIDS
- HORMONES/STEROIDS
- MYCOTOXINS
- NITRATES/NITROSAMINES
- PLASTICIZERS/PHTHALATES
- POLYAROMATICS/  
POLYCHLORINATED  
BIPHENYLS
- PROTEIN
- SUGARS/CARBOHYDRATES/  
SUGAR SUBSTITUTES
- FEED ADDITIVES/ARTIFICIAL  
FATTENERS
- FLAVORING/AROMA  
COMPOUNDS/CONSTITUENTS

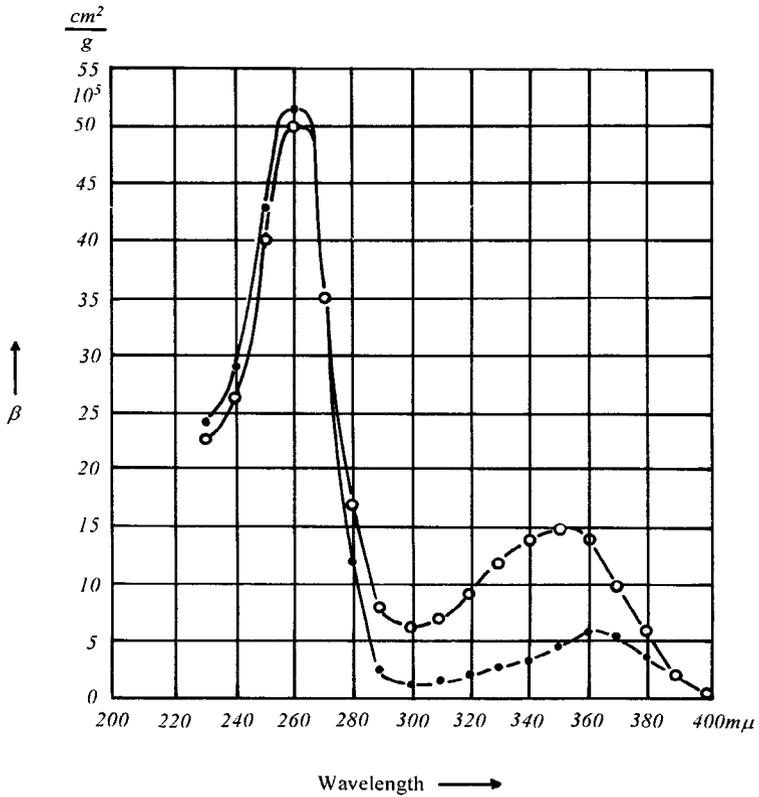
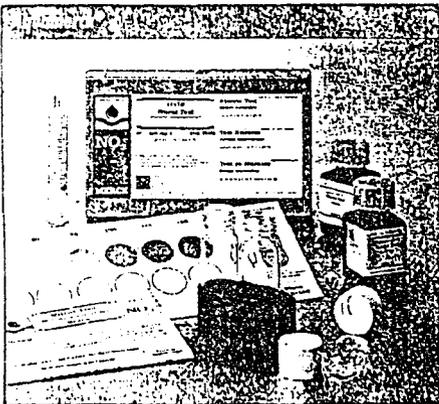
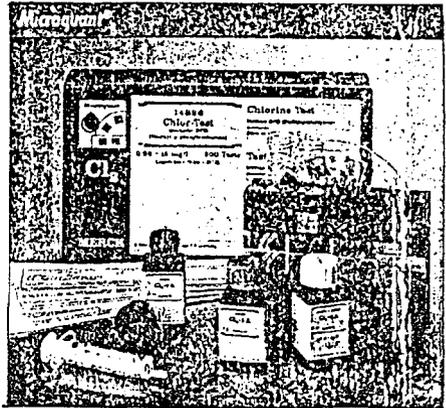
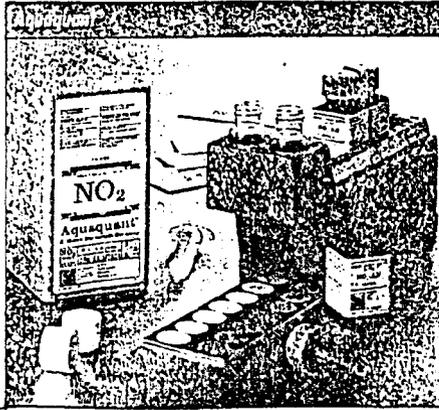


Figure 1. UV spectrum of "non-hydrogenated coferment"  $\text{NAD}^+$  (●) and "hydrogenated coferment"  $\text{NADH}$  (○), from the original paper by WARBURG et al. (Biochem. Z. 282, 157 [1935]).



Detailed product information indicates areas of application (e.g., water recovery, swimming pools, construction industry) and gives details about measuring ranges, methods, and number of determinations so that you can just pick the ready-to-use test for your application.



## **FOOD SAFETY - A VITAL ELEMENT OF FOOD SECURITY**

*Quintin L. Kintanar, M.D., Ph.D. and Carmina J. Parce, M.Sc.  
Bureau of Food and Drugs, Department of Health, Philippines*

### **ABSTRACT**

The key elements of food security are supply adequacy and physical and economic accessibility. Supply adequacy depends on the adequacy of food production but the equity of people's access to available food supply depends on the efficiency of distribution systems and purchasing capability. Thus, an adequate national food supply has to be supported by appropriate marketing facilities, equitable development policies, price stabilization mechanisms, and adequate income-generating opportunities. Moreover, adequate supply of food is not only a question of sufficient volume of production and access, but also of safety and quality of the food supply in order to sustain a healthy life.

In these times of rapid urbanization of a fast growing population, developments in agriculture and processing technologies to cope with the demands for increase in food production and changes in food preferences have brought to the fore a number of food safety issues that encompass microbiological and chemical contamination as well as safety concerns related to new products, processes and packaging. Various programs to address these food safety issues include: the Integrated Pest Management, Industrial and Agricultural Waste Management, Food Contamination Monitoring and Sanitation and Disease Control.

Food Security is defined by the Committee on World Food Security as the economic and physical access to food of all people at all times.

### **SUPPLY ADEQUACY, PHYSICAL AND ECONOMIC ACCESSIBILITY**

Three or four decades ago, food security concerns common to many developing countries were focused on recurrent food shortages. These shortages resulted from a variety of factors related to economic and technological backwardness. The slow growth of food output could not keep pace with the rapidly growing population. The situation was made worse by the vagaries of weather conditions which made agricultural production unpredictable. For many developing countries, reserve stocks were often inadequate or even nonexistent. Importation of food was the usual option. And when availability of foreign exchange was also a problem, these countries had to rely on food aid for relief.

At that time, national food security policies were aimed at overcoming food shortages with emphasis on early warning systems and appropriate food stocking policies.

The Food and Agricultural Organization (FAO) of the United Nations initiated programs for cooperation among developing countries to strengthen national efforts to meet food security needs and attain greater collective self-reliance.

These initiatives were geared towards: a) providing early warning of the advent of drought, crop failures, plant disease, etc.; b) enabling deficit countries to obtain imports more quickly from surplus countries; c) improving the bargaining position of developing countries in the international market; d) providing easier and more reliable access to food reserves within the region in times of shortage; and e) facilitating cooperative efforts among countries of the region in increasing production through transfer of technology. (1) The Philippines, as a developing country and FAO member state, is participating in some of these programs.

POVERTY is the root cause of food insecurity. Wherever there are extremely poor or seriously disadvantaged people in any country, developed or developing, food insecurity exists. It was estimated that more than 1 billion out of the total 5.5 billion people in the world live in poverty. Despite substantial increases in food production in many countries, millions of people still suffer from hunger or undernourishment. Food security encompasses not only factors relating to reliability of food production or its sustainability but also the equity of people's access to available food supplies. An adequate national food supply has to be supported with appropriate marketing facilities, equitable development policies, price stabilization mechanisms and adequate means of income-generating opportunities for the poor. (4) For the food regulatory authorities, the area of concern that is vital to food security is the safety of the food supply. When the safety of a major food item is in question, it can have a tremendous negative impact on its consumption and can result in serious health and economic consequences. For instance, the periodic occurrence of red tide bloom in certain coastal areas in Luzon and the Visayas have significantly reduced consumer demand for mussels and other shellfish which may accumulate in their bodies toxic levels of saxitoxin - the toxin present in the red tide microorganisms which are dinoflagellates such as the *Pyrodinium bahamense*. Losses in terms of actual income opportunities for shellfish producers and traders have exceeded a million pesos and put in distress thousands of poor people whose livelihood depended mainly on shellfish cultivation.

Another example is the aflatoxin contamination of peanuts due to poor post harvest handling practices. This has led to costly recalls of processed peanut products found exceeding the country's specified safety standards. The use of aflatoxin-contaminated corn meal in animal feeds can cause stunting of growth of livestock with corresponding economic consequences to agricultural productivity.

Food being a highly emotional issue, perceived lapses in food safety, real or imagined, command tremendous public health attention and concern.

The highly publicized "food scares" such as the ALAR spraying of apples in the United States, the cyanide tainted grapes from Chile, and just recently, here in the Philippines, the allegedly formalin treated vegetables, had devastating effects on the fruit growers and vegetable farmers.

Heightening the natural sensitivity of consumers to food safety issues are the overzealous media and sometimes irresponsible reporters blow up incidents of food poisoning or food contamination without verifying the real facts. As a

result, consumers are strongly influenced by "scare news". Unbalanced reports or statements taken out of context often find their way in print and broadcast media. It is not, therefore, surprising that public perception of food safety may be different from the opinion of experts in food science and/or toxicology.

## CURRENT FOOD SAFETY ISSUES

What are the food safety issues that may affect food security?

A review of the assessments of food control agencies in the health and agricultural sector, including reports of relevant UN agencies such as the World Health Organization (WHO) and FAO, show common areas of concern which can be grouped into three major clusters: 1) microbiological safety, 2) chemical residues and 3) new products/processes and packaging.

Microbiological Safety. Foodborne diseases remain one of the most common causes of morbidity and mortality throughout the world. Even in highly industrialized and developed countries with well-developed programs in sanitation and food handling practices, foodborne disease outbreaks still occur as documented by the U.S. Center for Disease Control. A considerable increase in the spectrum of diseases and corresponding causative foodborne pathogens were recognized over the past 15 years, from 1973 and 1987. Among these new pathogens that have been implicated in various foodborne disease outbreaks are *Campylobacter*, *E. coli* 0157:H7, *Yersinia enterocolitica*, *Listeria monocytogenes*, and new strains of *Vibrios* and *Salmonella*. (2) Varied susceptibility to foodborne pathogens became an area of concern as *Listeria monocytogenes* and *Vibrio vulnificus* were shown to cause much more serious illness in immune compromised individuals including pregnant women, unborn children, the elderly, AIDS patients, those suffering from cirrhosis of the liver, organ transplant recipients, and cancer patients receiving chemotherapy.

New concerns relate to increasing evidence that microorganisms may cause diseases in organ systems other than the gastrointestinal tract. Reactive arthropathy and rheumatoid disorders have been shown to be an aftermath of salmonellosis. *Listeria* can migrate to the blood causing septicemia and can lead to meningitis. Yersiniosis can produce subsequent arthritis and occasionally, septicemia and meningitis. *Vibrio vulnificus* causes wound infections and septicemia that can have a 50% fatality rate. And a serum urinary tract infection, hemolytic uremic syndrome which can lead to acute kidney failure, is caused by *E. coli* 0157:H7. (13)

Amidst all the concern about emerging foodborne pathogens and the non-gastrointestinal disorders caused by these organisms, the microbiological hazards posed by street vended foods need serious attention by local health officials. Street food vending in developing countries is a growing business that has gained the patronage of many people because street foods are available at affordable

prices and in conveniently located areas, both in urban and rural communities.

A recent memorandum from government epidemiologists of the Department of Health (DOH-FETP) pointed out that a number of studies provide evidence that eating street foods is a risk factor for enteric disease transmission. (3)

**Chemical Residues.** The next cluster relates to chemical residues from pesticides, environmental contaminants, and animal drugs. These are the contaminants that are on top of the list of potential hazards associated with food as perceived by most consumers. Much of the apprehension is probably due to their lack of understanding of the complexity of scientific, toxicological and agricultural issues involved, and perhaps their limited knowledge of how these contaminants are controlled by responsible authorities. Any reported presence of chemical residues in food, regardless of the level found, seem to cause anxiety as consumers are not in a position to determine how much residue present in a particular commodity is still considered safe or acceptable. This leads to indecision whether or not to buy or consume a suspected product and the resultant fear and apprehension in the public mind.

In many developing countries, agricultural chemicals are considered indispensable to raise crop yields and improve farmers' economic security. However, misuse of pesticides pose a serious health threat not only to handlers and farmers, who are either ignorant or deliberately disregard precautions in safe handling of the pesticides, but also to environmental quality.

Highly toxic metallic substances such as lead, cadmium and mercury are some of the environmental pollutants derived from industrial emissions. When deposited in the soil or carried into bodies of water, these can eventually contaminate plants, fish and other edible aquatic materials. (Figure 1) Appropriate preventive or ameliorative measures may be taken at various levels. (Figure 2)

Historically, the mercury contamination of Minamata Bay in Japan killed and disabled hundreds of people due to the cumulative rise in mercury levels in fish that reached 20 ppm (or 20 mg/kg). In many developing countries, use of gasoline with high lead content is a leading cause of environmental pollution. Lead can also come from lead soldered metal containers used in packaging food. On the other hand, cadmium-containing enamel and ceramic glazes and cadmium-based pigments or stabilizers in plastics may be ultimate sources of food contamination. (9) Other toxic chemicals which can find their way into our food include polychlorinated biphenyls (PCBs) which are widely used in dielectric fluid transformers and capacitors. Other applications are in heat transfer and hydraulic fluids and in the formulation of lubricating oils, and as plasticizers in paints, copying paper and other products. (11) Contamination of edible oil with PCBs led to large scale intoxication incidents in Japan in 1968 and Taiwan in 1979. (12) Limited evidence of carcinogenicity to humans was obtained from the incident in Japan as well as from studies of occupationally-exposed populations. However, the International Agency for Research on Cancer (IARC) reports sufficient evidence of PCB carcinogenicity in experimental animals and, therefore, PCB is regarded as a potential carcinogen in humans. (5) Recent evi-

dence also suggested that transplacental exposure to PCB may result in developmental delay with manifested effect on the cognitive functioning in young children. (7)

**New Products/Processes and Packaging.** Increasing demands are placed upon global food processing and distribution systems as a result of rapidly growing and an increasingly urbanized world population. This has great implications for the food supply.

The use of microwaves under pressure, ultra-high-temperature food processing, and continuous computer control of pasteurization systems are examples of new approaches to food processing that require safety evaluation.

Biotechnology and its applications to production of new food ingredients have been explored in the past twenty years. The first food-related application of genetic cell modification was in the production of the enzyme chymosin from *E. coli* K12 for the manufacture of cheese. The next group of products is expected to be foods from transgenic plants. Gene transfer has been used to produce corn, potato, and tomato crops that are resistant to herbicides and certain insects. (5)

There are concerns that expression of the transferred genes may produce unexpected, possibly allergenic effects, or changes in nutrient/toxicant ratios. Prospective users of food biotechnology face the following issues: a) assurance of the purity and stability of microbiological cultures used in processes; b) possible consumer exposure to microbial metabolites and foreign DNA; and c) difficulty in assessing possible levels of exposure to such compounds, as consumption of these products increases or changes. (8) Technological advances has brought about remarkable improvements in packaging materials for high quality, shelf-stable, fresher tasting and safer food products. However, new safety issues may arise on the use of improved films and trays with modified atmosphere conditions for fresh or minimally processed food such as extended shelf-life refrigerated products. The quality of polymerized substances in plastics/laminates and its possible migration into the packaged food is still an area of concern.

The appearance of low-fat, low-cholesterol, and low-calorie substitutes for macronutrients may bring about additional food safety issues. The potential increase in consumption of these so-called "designer food" ingredients, displacing more traditional dietary staples, is becoming a concern to nutrition advocates.

## PROGRAMS FOR FOOD SAFETY ASSURANCE

National efforts to address food safety issues are varied. The more significant control measures are as follows:

**The Integrated Pest Management Program** is the approach for the effective prevention and control of pesticide residues in food. The program aims to instill responsibility in chemical handlers and farmers to properly use pesticides to protect the health of consumers and the quality of the environment for

sustainable development.

**The Program for Industrial and Agricultural Waste Management** is needed to arrest further degradation of agricultural lands and water environment that are sources of our food supply.

**The Food Contamination Monitoring Program** is a strategic means to provide data on levels and trends in food contamination that can be used as basis for preventive regulations. Aside from protecting domestic consumers, it strengthens a country's position in international markets by ensuring the quality of exported food. The UNEP/FAO/WHO Food Contamination Monitoring Program is a global environment monitoring system for collecting data on food contamination through a network of participating institutions in more than 60 countries worldwide.

**Programs for Sanitation and Disease Control** to address microbiological contamination of food, range from approaches to dealing with on-farm sources of pathogens to improvements in sanitation and food handling practices in the home. Animal husbandry approaches should be sought to deal with on-farm sources of foodborne pathogens through improved sanitation, vaccination or breeding of resistant varieties of animals. Effective measures to control infections in food animals would complement sanitation efforts at the slaughtering and processing stage.

In addition to avoiding pathogen contamination of raw materials, efforts should also be directed towards elimination of pathogens by food processing, control of pathogen growth in foods and prevention of recontamination during storage and distribution. The Committee on Food Hygiene of the Codex Alimentarius Commission has endorsed the adoption of the Hazard Analysis Critical Control Point System (HACCP) for the food industry as an effective and rational means of ensuring food safety, HACCP is a systematic approach that will underscore the food industry's role in continuous prevention and control of food safety problems and departs from reliance on traditional facility inspection by regulatory agencies to detect loss of control.

## CONCLUDING STATEMENT

Man needs food to live but having adequate supply and access to food alone does not ensure ones health and well-being. The ultimate goal of food security is for man to have access to adequate supply of safe and good quality food needed to sustain a healthy life.

Let us not forget that assurance of food safety is a shared responsibility of the food industry, the various government regulatory authorities, and the most

important of all, the consumer who will have to make the final decision on what and how much food to eat.

#### REFERENCES:

1. Approaches to World Food Security. 1983. FAO Economic and Social Development Paper 32.
2. Bean N H and Griffin P M. 1990. Foodborne disease outbreaks in the United States, 1973-1987: Pathogens, vehicles and trends. *J Food Protection* 53:804-817
3. Dayrit, M M, Magboo, F L and Mateo, R J. 1994. Completed studies establishing the association between enteric diseases and eating food bought from street food vendors. FETP Memorandum. Department of Health
4. Food Security For All. 1992. Food and Nutrition: Creating a well fed world. FAO World Food Day Secretariat. FAO Rome
5. Harlander S K. 1991. Biotechnology - a means for improving our food supply. *Food Tech* 45(4):84-95
6. IARC 1987. IARC monographs of the evaluation of carcinogenic risk to humans. Supplement 7
7. Jacobson J L, Jacobson S W and Humphrey H E. 1990. Effects of in-utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. *J Pediatr* 166(1):38-45
8. Miller S A. 1992. Novel Foods: Safety and Nutrition. *Food Tech* 46(3):114-117
9. UNEP/FAO/WHO. 1988. Assessment of Chemical Contaminants in Food. UNEP, Nairobi
10. UNEP. 1992. The Contamination of Food. UNEP/GEMS Environmental Library No. 5. UNEP, Nairobi
11. Van der Kolk J. 1984. Consideration of a CODEX approach to contamination of foodstuffs with PCBs. CX/PR 84/10. Codex Alimentarius Commission Committee on Pesticide Residues
12. WHO. 1987. PCBs, PCDDs and PCDFs: Prevention and control of accidental and environmental exposures. Copenhagen, WHO Regional Office for Europe

13. Wolf I D and Lechowich R V. 1989. Current issues in micro-biological food safety. *Cereal Foods World*. 34(6): 468-471

# NUTRITION CONSIDERATIONS IN FOOD SECURITY: THE CASE OF THE PHILIPPINES

*Rodolfo F. Florentino, M.D., Ph.D.  
Food and Nutrition Research Institute  
Department of Science and Technology*

## ABSTRACT:

Nutritional considerations dictate that the average food intake of the population conform to the Recommended Dietary Allowance (RDA) defined for that population. For purposes of food and nutrition planning, however, particularly planning for food security, the quality of food supply expressed in the form of a Desirable Dietary Pattern (DDP) has to be taken into account. Applying these norms to the Philippines, it appears that deficiencies apparently exist not only in total food supply but in some major food groups. Employing the DDP further beyond the Year 2000, and assuming particular levels of dietary energy supply, one can arrive at estimates of food supply requirements for the country to attain food security in the coming decades.

## INTRODUCTION

Nutritional considerations dictate that the average food intake of the population conform to the Recommended Dietary Allowance (RDA) defined for that population. For purposes of food and nutritional planning, however, particularly planning for food security for the country as a whole and its subdivisions, the physiological requirements embodied in the RDA has to be translated into quantities of food and food groups that the country has to either produce for its population or import from outside. Thus in order to attain nutritional adequacy for national security, consideration has to be given both to the quantity of food supply (based on the RDA plus allowance for losses and distributional differences) and the nutritional quality of food supply (based on a desirable dietary pattern that takes nutritional considerations as well as cultural patterns of eating into account). This paper illustrates the use of the RDA and the desirable dietary pattern (DDP) for the estimation of food supply requirements at the national level, using the Philippines as a case study.

### Recommended Dietary Allowances

Every individual requires a certain amount of dietary energy and essential nutrients to maintain its normal functions and for proper growth and development. A minimum level (called Minimum Dietary Requirement or MDR) is required below which manifestations of malnutrition appear. Such manifestations

include loss of weight, poor growth, stunted mental development, lowered resistance to infection, diminished physical capacity and lowered physical and mental performance, not to mention physical lesions in various parts of the body.

Individuals differ in their MDR not only because of differences in age, sex, physical activity and body size but because of biological variability. In order to take care of individual variations, an allowance or margin of safety is added (except in the case of dietary energy) to average MDRs of individuals of a given age, sex, physical activity and body size, to come out with what is called Recommended Dietary Allowances (RDA).

In general, intakes above the RDA will take care of the needs of practically all of the healthy population. However, it does not necessarily mean that individuals with intakes below the RDA will develop nutritional deficiency because of the margin of safety added to minimum requirements. It simply means that the risk of nutritional deficiency increases as the intake of individuals decreases below the RDA.

In the case of energy, the RDA represents the average requirement of the population, and a margin of safety is not added. Thus populations whose average energy intake is below the RDA are likely to reduce their energy expenditure or are stunted in growth and development to compensate for their low energy intake.

The RDA is most useful for assessing the nutritional adequacy of intakes of population groups, but it is also useful in assessing the nutritional adequacy of food supplies for a given population. Conversely, the RDA may also be used as a guide in prescribing adequate diets of individuals and groups, as well as in planning adequate food supplies for the population.

For the Philippines, the Food and Nutrition Research Institute (FNRI) came out in 1989 with the latest revision of Philippine RDA for various age and physiologic groups (7). (Table 1) The levels assume a moderate level of physical activity and normal body weight. Taking the current Philippine demographic structure, the average RDA for the population for the different nutrients could be estimated. Using the 1990 population structure, the average RDA for energy is 1993 kilocalories and that for protein is 49.9 grams per capita per day. (Table 2) The 1990 Philippine RDA also recommends the desirable level of carbohydrate amounting to 55-70% of total calories, protein, 10-15%, and fat, 20-30%.

### **Desirable Dietary Pattern for Food and Nutrition Planning**

For purposes of food and nutrition planning particularly for populations, the physiological requirements embodied in the RDA need to be translated into food and food groups. This, however, needs to take into account the customary patterns of eating in addition to the RDA. Taking both of these parameters into account (the RDA and the customary pattern of eating), a recommendation can be made for a desirable combination of food groups as an estimate of nutritional requirements in terms of various foods rather than in terms of nutrients. The resulting recommendation, called Desirable Dietary Pattern (DDP), could there-

fore be used both as a yardstick for the quality of a given diet and for estimating a quantitative target for food and nutrition planning. Thus the average diet of population groups or the pattern of available food supply could be compared with the DDP to arrive at a qualitative score of the diet or set of foods. Using the DDP and assuming a target quantity of dietary energy available for consumption, food supply targets for the country or its regions could be estimated for the various food groups.

Based on the Philippine RDA and customary pattern of food intake of Filipinos obtained from the 1987 National Nutrition Survey (Table 3), the FNRI has arrived at a recommended DDP for food and nutrition planning (Table 4) (1). The DDP recommends that cereals should take up 53% of total calories, animal products, 18%, added fat, 10%, and fruits and vegetables, 5%, and so on. If a diet or set of foods conform to the recommended pattern, such a diet or set of foods would have a score of 100.

### **Food Demand**

At the same time as the food supply requirements of the country are estimated from the RDA and the DDP, it is best to study the historical food demand of the population in order to determine how these estimates of food supply requirements thus obtained compare with actual food demand.

The actual food demand of the population could be estimated from two sources: the Food Balance Sheet and Food Consumption Surveys.

#### **1. Food Balance Sheet**

The Food Balance Sheet (FBS) gives estimates of supply of the different food groups available for consumption for a given year. These estimates are derived by subtracting food losses, non-food uses and exports from the total food produced in the country, plus imports and beginning stocks.

While the FBS is primarily an estimate of food supply available for consumption, it gives some measure of the amount of food demand of the population in a given year. Moreover, the estimates of available food supply could be translated into quantities of dietary energy and nutrients and compared to the recommended allowances to give an assessment of nutritional quality of the food supply.

However, the FBS is an aggregate for the entire country. It does not take into account inequalities in distribution among geographic areas, among population groups, and among families and individuals. While post-harvest losses from farm to market are imputed, losses from market to household and individual levels are inadequately covered.

In the case of the Philippines, trends in food supply show that total food supply available for consumption on an annual per capita basis increased from

1057.4 grams in 1978 to 1067.8 grams in 1992 (Table 5) (4, 5, 6). Despite some decline from calendar year 1985 to 1987, the country managed to maintain adequate foodstuff to meet the requirements of its growing population.

The food supply situation shows that the bulk of total food net supply was of vegetable origin. During the period, the share of vegetable-based food items to the total net supply increased from 78 percent in 1978 to 82 percent in 1992.

Over the years, available supply of cereals, sugars and syrups, pulses and nuts have increased, while starchy roots and tubers, fats and oils and miscellaneous food declined. Vegetable and fruit supply practically remained steady. On the other hand, supply of animal based food items, as a whole, decreased except for meat, poultry and fish which apparently exhibited increases over the years since 1978.

In terms of nutritive values, per capita calorie and protein supply were apparently adequate compared to recommended allowances. On the average, calorie supply per capita per day reached 2501 kilocalories in 1992 from 2208 in 1978, exceeding the average requirement of 1993 kcal per capita per day by 25.5 percent (Figure 1 and Table 5). There was a decline in per capita calorie supply from calendar years 1983 to 1987, followed by a sharp rise since then. The per capita protein supply in 1992 was greater than the RDA (49.9 grams) by 37.1 percent (Figure 2 and Table 5). As in caloric supply, there was a sharp rise in protein supply after 1987 following the fall during the mid-eighties.

Based on the recommended DDP, the dietary score of the food supply for the country has remained between 76 to 78 for the period 1978 to 1992 (Table 6). Thus at the same time that the per capita food supply barely kept pace with the increasing population, the quality of food supply has also not been significantly improving. In fact it has been deficient in animal products, added fat and pulses and beans.

## 2. Food Consumption

Food consumption surveys provide a more accurate estimate of actual food demand of the population at a given time since it is based on food intake measurements of households and individuals.

The food consumption surveys of FNRI uses the one-day household food weighing method (8). All foods to be prepared and intended for the whole day's consumption including snacks are weighed. Also accounted for are food wastage in every meal and left overs of the day's consumption. Food items weighed include the following: a) raw "as purchased" foods to be cooked for each meal, b) foods served and eaten raw, c) cooked or processed foodstuffs served directly on the dining table, and d) non-perishable items such as coffee, sugar, cooking oil and the like. Meals and snacks eaten outside the home by the household members are recalled and recorded as part of the intake of the household for the day.

The national nutrition surveys covered a total of about 3000 household samples on the average per reference survey year. Selection of samples was on

the basis of a stratified three-stage sampling design. All regions in the country are included and the provinces, barangays and households serve as sampling units.

The food consumption survey of FNRI from 1978 to 1987 showed that the Filipino diet consisted mainly of rice and fish with some succulent vegetables (8). Over the years, intake of cereals declined significantly from 367 grams per capita per day in 1978 to 345 grams in 1987 due to the continuing decrease primarily in the consumption of corn and corn products (Table 7). The consumption of starchy roots and tubers, on the other hand, showed an increase in 1982 followed by a drastic drop in 1987. Consumption of animal products improved from 182 g in 1978 to 210 g in 1987. Fish and marine products accounted for more than half of the total consumption of animal products. Per capita intake of added fat, mostly cooking oil, remained almost unchanged at 12g per day over the three survey periods. Nuts and oilseeds were consumed the least while consumption of sugar gradually increased over the same period. The daily per capita consumption of fruits and vegetables declined continuously during the three survey periods. The consumption of beverages like coffee, cocoa, "tuba" (coconut toddy), and seasonings like salt and vinegar, revealed varying trends.

In terms of energy, protein and other nutrients, the average daily per capita intake had been found adequate only for niacin (Table 8). The main nutritional problem over the years remained to be energy inadequacy. Energy intake which was maintained in 1978 and 1982 at more than 1800 kcal declined to 1753 kcal in 1987, a 3.0% decrease. These energy intakes showed wide gaps from the RDA of 11.4%, 11.0% and 12.9% for 1978, 1982 and 1987, respectively. On the other hand, protein intake was found to be close to average recommended amount although it was still short of being adequate. Persistent mineral and vitamin inadequacies was also revealed. Thus, serious short fall in the dietary intake of riboflavin, thiamin and calcium was noted. The repercussion of the economic crisis in 1983 - 1985 affected the trend in nutrient intake which manifested significant decline in the diet as shown in the 1987 survey. With some improvements in the country's economy after this period, improvement in the diet can be expected to follow. Hopefully the 1993 food consumption survey for which field data collection has just been completed will reveal improvement in food consumption.

Based on the recommended DDP, the dietary score of the average food intake of Filipinos (Table 9) has improved slightly since 1978 (dietary score, 80.5; 1982, 80.0; 1987, 82.6). Again it appears that while the quality of the diet has been improving albeit slowly, the total quantity of dietary energy, not to mention other important nutrients such as vitamin A, iron, thiamin and riboflavin, has been inadequate compared to RDA.

Comparing the 1987 FBS with the 1987 food intake data, we note that in spite of 2284 kcal per capita available supply for consumption per day, the average energy intake of the population was only 1753 kcal which was only 87% adequate. In the case of protein, the 1987 per capita available supply was 65.6 g while actual average intake was 49.7 g which 98% adequate.

These differences could presumably be accounted for by inequalities in distribution and in non-accounted food losses. We would therefore probably

require more than 30% of available energy supply above energy requirements in order to attain energy adequacy on the average among the population assuming other factors being constant.

### 3. Malnutrition and Nutrition Deficiencies

As a direct consequence of inadequate dietary intake described above, malnutrition and various forms of specific nutritional deficiencies abound.

Generalized malnutrition resulting from energy and protein lack and manifested by underweight, stunting and wasting, has been found in a significant proportion of children in all FNRI surveys (8.9). The latest 1993 anthropometric survey conducted by FNRI revealed 8.4% of preschool children who were found moderately underweight, 5.6% were stunted indicating chronic malnutrition, and 6.2% were wasted indicating acute malnutrition (Figure 3)(9).

The 1993 FNRI surveys also showed significant problem of nutritional anemia (Figure 4), especially among infants (49.2%) pregnant (43.6%) and lactating women (43.0%), and the elderly (45.6%)(2). Goiter which is a manifestation of iodine deficiency was also high especially among pregnant and lactating women (Table 10). (3)

### Food Supply Requirements Based on DDP and RDA

The above data on food consumption and malnutrition rates indicate that in order to attain nutritional adequacy for national security, we have to consider both the quantity of the available food supply (as measured by total dietary energy available for consumption) and quality of food supply (as measured by the DDP)

Using the recommended DDP for the Philippines, various levels of nutritionally desirable targets of available supply for the different food groups could be estimated assuming various targets of available energy supply. Thus targeting 2600 kcal per capita per day as available energy supply (based on the RDA of 2000 kcal per capita per day and adding 30% margin for losses and inequalities in distribution), the DDP recommends a food supply requirement for the various food groups shown in Table 1 from the year 1995 to year 2020.

Thus for 1995, the recommended food supply requirement that should be made available for consumption for the various food groups would be as shown in Table 11. Comparing this with the available supply in 1992, it would appear that while the level of production of cereals is more or less adequate, there is a need to greatly increase the supply level for animal products, added fat, and pulses and beans, not only to keep pace with population increase but to improve the quality of the diet.

Assuming higher targets of calorie supply as further hedge against nutritional inadequacies among the vulnerable groups in the population, the food supply requirements would be as in Tables 12 and 13. Table 12 gives the estimated food supply requirement assuming a target supply of 2700 kcal per capita per day

and using medium population projection. Table 13 gives the estimated food supply requirement assuming a target supply of 2800 kcal.

Depending therefore on food supply policies adopted by the country and depending upon the targeted calorie supply, the attainment of food security based on nutritional needs could be realized to a lesser or greater degree.

### CONCLUSION

In order to attain nutritional adequacy for national food security, both the quantity (primarily based on calorie and protein adequacy relative to RDA) as well as the quality (based on a desirable dietary pattern or DDP) of the food supply has to be taken into account.

The Philippines has arrived at its own recommended DDP considering nutritional requirements and cultural patterns of eating. Based on the recommended DDP, there is apparently a need for the Philippines to greatly increase its supply level for animal products, added fat and pulses and beans not only to keep pace with population increase but to improve the quality of the diet.

Assuming particular targets of calorie supply and population increase, the estimated food supply requirements of the Philippines for various food groups could be estimated for the ensuing years.

### REFERENCES:

1. Florentino, R.F. 1991. Proposed Desirable dietary Pattern. Report of the Workshop on the Formulation of Dietary Guidelines for Food and Agriculture Planning, November, 1990. National Nutrition Council. 2. Kuizon, M. D., et al. 1994. Fourth National Nutrition Survey, Philippines: 1993. Part D. Biochemical Survey. Food and Nutrition Research Institute-DOST.
2. Magbitang, J.A., et al. 1994. Fourth National Nutrition Survey: Philippines: 1993. Part C. Clinical Survey. Food and Nutrition Research Institute-DOST.
3. National Statistical Coordination Board. 1988. The Philippine Food Balance Sheet CY 1973-1986.
4. National Statistical Coordination Board. 1990. The 1987-1989 Food Balance Sheet of the Philippines.
5. National Statistical Coordination Board. 1993. The 1990-1992 Food Balance Sheet of the Philippines
6. RDA Committee. 1989. Recommended Dietary Allowances for Filipinos, 1989 Edition. Food and Nutrition Research Institute-DOST.

7. Villavieja, G.M. 1991. Food Consumption Situation and Nutritional Problems in the Philippines. Report of the Workshop on the Formulation of Dietary Guidelines for Food and Agriculture Planning, November 1990. National Nutrition Council.
8. Villavieja, G.M. et al. 1994. Fourth National Nutrition Survey, Philippines: 1993. Part B. Anthropometric Survey. Food and Nutrition Research Institute-DOST.

**Table 1. Recommended dietary allowances for Filipinos for energy and specific nutrients.**

(Average Per Day)  
1990 Edition

Designed to maintain health and provide reasonable levels of reserves in body tissues of  
nearly all healthy persons in the population

Age (y)	Weight kg	Energy kcal	Protein g	Vit. A ugRE	Vit. C mg	Thiamin mg	Riboflavin mg	Niacin mgNE	Folate ug	Calcium mg	Iron mg	Iodine mg
<b>Infants</b>												
3 - < 6 mo	6	620	a	325	30	0.3	0.3	5	20	300	10	40
6 - < 12 mo	9	880	14	325	30	0.4	0.4	8	30	400	15	50
<b>Children</b>												
1 - 3	13	1350	27	350	354	0.7	0.7	13	40	600	9	55
4 - 6	18	1600	32	375	45	0.8	0.8	15	60	600	10	65
7 - 9	24	1740	35	400	55	0.9	0.9	17	80	600	12	70[C1]
<b>Males</b>												
10 - 12	32	2090	45	425	65	1.0	1.0	20	100	700	16	85
13 - 15	44	2340	60	475	75	1.2	1.2	22	140	700	18	105
16 - 19	55	2580	69	525	90	1.3	1.3	25	170	700	17	120
20 - 39	56	2570	60	525	75	1.3	1.3	25	170	500	12	120
40 - 49	56	2440	60	525	75	1.2	1.2	23	170	500	12	120
50 - 59	56	2320	60	525	75	1.2	1.2	22	170	500	12	120
60 - 69	56	2090	60	525	75	1.0	1.0	20	170	500	12	120
70 +	56	1880	60	525	75	0.9	0.9	18	170	500	12	120
<b>Females</b>												
10 - 12	35	1910	49	400	70	1.0	1.0	18	110	700	17	80
13 - 15	44	2010	56	425	75	1.0	1.0	19	140	700	(21) <sup>b</sup>	100
16 - 19	48	2020	56	450	80	1.0	1.0	19	150	700	(25) <sup>b</sup>	100
20 - 39	49	1900	52	450	70	1.0	1.0	18	150	500	(26) <sup>b</sup>	100
40 - 49	49	1800	52	450	70	0.9	0.9	17	150	500	(26)	100
50 - 59	49	1710	52	450	70	0.8	0.8	16	150	500	11	100
60 - 69	49	1540	53	450	70	0.8	0.8	15	150	500	11	100
70 +	49	1390	52	450	70	0.7	0.7	13	150	500	11	100
<b>Pregnancy</b>												
1st trimester												
2nd trimester			[ +9	[ +25	[ +10	[	[	[	[ +200	[ +400	[ (41) <sup>b</sup>	[ +25
3rd trimester		[ +300	[	[	[	[ +0.3	[ +0.6	[ +3	[	[	[	[
<b>Lactation</b>												
1st 6 mo		[ +500	[ +16	[ +325	[ +35	[ +0.4	[ +0.4	[ +5	[ +100	[ +400	[ (23) <sup>b</sup>	[ +50
2nd 6 mo		[	[ +12	[ +275	[ +30	[	[	[	[	[	[	[
<b>Average</b>	<b>1993</b>	<b>49.9</b>	<b>459.0</b>	<b>66.9</b>	<b>1.02</b>	<b>1.02</b>	<b>19.0</b>	<b>134.3</b>	<b>587.9</b>	<b>17.6</b>	<b>96.8</b>	

<sup>a</sup> Will be met if energy needs are met. See text

<sup>b</sup> These recommended dietary allowances cannot be met by the usual diet, thus, supplementation is recommended.

**Table 2. Recommended dietary allowances for Filipinos<sup>1</sup>.**

Nutrient	Average Per Capita Per Day
Energy (kcal)	1993
Protein (g)	49.9
Vitamin A ( $\mu$ g RE)	459.0
Vitamin C (mg)	66.9
Thiamin (mg)	1.02
Riboflavin (mg)	1.02
Niacin (mg NE)	19.0
Calcium (mg)	587.9
Iron (mg)	17.6
<b>% Total Calories</b>	
Carbohydrate	55 - 70
Protein	10 - 15
Fat	20 - 30

<sup>1</sup> 1989 Edition [7]

**Table 3. Pattern of food intake.1987.**

Food Group	% Cal	kCal	G/Cap/D
Cereals	69.2	1213	344.6
Roots and Tubers	1.3	23	21.1
Animal Products	11.6	203	207.5
Added Fat	5.2	91	11.7
Nuts and Oilseeds	1.1	19	7.9
Pulses and Beans	1.5	26	21.2
Sugar	4.8	84	23.4
Fruits and Vegetables	4.3	75	221.7
Beverage and Seasonings	1.0	1753	27.8
<b>TOTAL</b>	100.0	1753	896.9
Nutrient	% Cal	G/Cap/D	
Carbohydrate	74.1	313	
Protein	11.0	49.7	
Fat	14.9	30.0	

Table 4. Recommended desirable dietary pattern for the Philippines.

Food Group	kCal %	Rating	Score	Maximum Score
Cereals	53	0.5	26.5	50/40
Roots and Tubers	4	0.5	2.0	20
Animal Products	18	2.5	45.0	50
Added Fat	10	1.0	10.0	10
Nuts and Oilseeds	1	1.0	1.0	10
Pulses and Beans	3	1.5	4.5	20
Sugar	4	0.25	1.0	5
Fruits and Vegetables	5	2.0	10.0	10
Beverage and Seasonings	2	0	0	
<b>TOTAL</b>	<b>100</b>		<b>100.0</b>	

Table 5. Daily per capita food supply: Philippines, 1978-92.

Food Group	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992
in grams															
Total Per Capita Food Supply	1057.4	1077.5	1126.2	1113.1	1165.6	1137.9	1183.9	1025.1	1049.6	1047.5	1073.6	1104.1	1091.6	1024.9	1067.8
A. Vegetable Origin	829.3	864.5	899.8	880.2	925.4	891.3	944.1	798.0	817.4	871.8	890.5	913.1	898.4	837.3	875.6
1. Cereals	303.3	319.9	339.9	340.0	348.7	319.2	336.7	329.0	330.1	340.6	359.4	377.3	379.7	348.1	364.9
2. Starchy Roots/Tubers	98.1	99.0	91.5	93.7	81.2	60.5	64.3	63.6	62.9	71.6	76.1	68.4	65.3	62.5	61.6
3. Sugars/Syrups	29.6	32.1	29.6	23.8	27.6	27.1	30.9	25.2	26.1	40.6	49.4	54.8	61.7	58.3	62.4
4. Pulses/Nuts	11.5	10.3	12.0	11.5	10.9	9.6	7.8	10.1	12.0	25.0	23.3	28.9	29.2	22.0	22.0
5. Vegetables/Fruits	248.5	277.0	291.5	256.5	285.2	283.2	263.1	241.2	242.5	282.0	273.4	276.5	256.2	244.3	262.1
6. Fats and Oils	18.3	15.3	16.7	14.7	13.3	11.7	10.6	10.8	12.0	14.7	15.2	14.9	14.8	14.6	15.0
7. Miscellaneous	120.0	110.9	118.6	140.0	158.5	180.0	230.7	118.1	131.8	97.3	93.7	92.4	91.5	87.5	87.6
B. Animal Origin	228.1	213.0	226.4	232.9	240.2	246.6	239.8	227.1	232.2	175.7	183.2	191.0	193.2	187.6	192.2
1. Meat/Poultry/Fish/ Marine Products	137.6	125.3	137.9	144.6	115.1	157.6	151.9	140.7	146.6	160.5	169.2	176.0	177.8	171.5	175.9
2. Milk	75.4	74.8	75.9	74.8	75.6	75.1	44.8	75.1	74.3	7.7	6.4	7.0	7.3	7.7	7.6
3. Eggs	15.1	12.9	12.6	13.5	13.5	13.9	13.1	11.3	11.3	7.5	7.7	8.2	8.1	8.4	8.7
Per Capita Calorie Supply	2208.0	2255.7	2373.4	2374.3	2442.5	2320.5	2373.6	2248.3	2308.3	2284.2	2414.6	2500.2	2561.2	2406.2	2501.1
RDA (1993 kcal)	110.8	113.2	119.1	119.1	122.6	116.4	119.1	112.8	115.8	114.6	121.2	125.4	128.5	120.7	125.5
PerCapita Protein Supply	61.3	61.7	65.5	66.6	68.9	67.4	66.3	64.4	65.6	61.5	65.0	68.6	69.9	66.7	68.4
RDA (49.9 g)	122.8	123.6	131.3	133.5	138.1	135.1	132.9	129.1	131.5	123.2	130.3	137.5	140.1	133.7	137.1

Table 6. Dietary score of food supply using recommended DDP 1987, 1990 and 1992.

Food Group	1987		1990		1992	
	% Cal	Score	% Cal	Score	% Cal	Score
Cereals	54.3	27.2	55.9	28.0	55.5	27.8
Roots and Tubers	4.3	2.2	3.6	1.8	3.4	1.7
Animal Products	9.7	24.3	9.9	24.8	10.2	25.5
Added Fat	5.5	5.5	4.9	4.9	5.1	5.1
Nuts and Oilseeds	2.0	2.0	1.8	1.8	1.4	1.4
Pulses and Beans	0.5	0.8	0.9	1.4	0.8	1.2
Sugar	6.7	1.7	9.1	2.3	9.5	2.4
Fruits and Vegetables	7.1	14.2	5.6	11.2	6.0	12.0
Beverage and Seasonings	9.9	0	8.3	0	8.1	0
<b>Total</b>	<b>100.0</b>	<b>77.9</b>	<b>100.0</b>	<b>76.2</b>	<b>100.0</b>	<b>77.1</b>

**Table 7. Trend in mean one-day per capita food consumption: Philippines, 1978, 1982 and 1987.**

Food Group	Consumption <sup>1</sup> g/day			% Increase/ (Decrease)	
	1 9 7 8	1 9 8 2	1 9 8 7	1978 vs 1982	1982 vs 1987
Cereals	367	356	345	( 3.0)*	( 3.1)*
Rice and Products	308	304	303	( 1.3)	( 0.3)
Corn and Products	38	34	24	(10.5)	(29.4)*
Bread and Other Cereals	21	18	18	(14.3)*	-
Starchy Roots and Tubers	37	42	22	13.5 *	(47.6)*
Animal Products	182	208	210	14.3 *	1.0
Fish and Marine Products	102	113	111	10.8 *	( 1.8)
Meat and Products	23	32	36	39.1 *	15.6 *
Poultry and Products	7	10	10	42.8 *	(10.0)
Eggs	8	9	10	12.5	11.1
Milk and Products	42	44	43	4.8	( 2.3)
Added Fat	11	12	12	9.1 *	-
Nuts and Oilseeds	9	8	6	(11.1)	(25.0)
Pulses and Beans	19	21	19	10.5	( 9.5)*
Sugars	19	22	24	15.8 *	9.1 *
Fruits and Vegetables	237	219	209	( 7.6)	( 4.6)
Vitamin C-Rich Fruits	30	18	24	(40.0)*	33.3 *
Other Fruits	74	83	82	12.2 *	( 1.2)
Green Leafy & Yellow Vegetables	34	37	29	8.8	(21.6)*
Other Vegetables	99	80	74	(19.2)*	( 7.5)*
Beverages and Seasonings	21	32	26	52.4 *	(18.7)*
Beverages	9	19	15	111.1 *	(21.0)*
Seasonings	11	12	11	9.1 *	( 8.3)*
Other Miscellaneous Foods					

<sup>1/</sup> Raw, as purchased value excluding edible wastage

\* Statistically significant

**Table 8. Mean one-day per capita nutrient intake and percent adequacy: Philippines, 1978, 1982 and 1987.**

Nutrient and Particulars	1978	1982	1987	% Increase/(Decrease)	
				1978 vs 1982	1982 vs 1987
Energy					
Intake (kCal)	1804	1808	1753	0.2	(3.0)*
% Adequacy	88.6	89.0	87.1		
Protein					
Intake (kCal)	48.0	50.6	49.7	5.4 *	(1.8)*
% Adequacy	93.2	99.6	98.2		
Iron					
Intake (kCal)	10.6	10.8	10.7	1.9	(0.9)
% Adequacy	88.3	91.5	91.5		
Calcium					
Intake (kCal)	0.44	0.45	0.42	2.3	(6.7)*
% Adequacy	78.6	80.4	75.0		
Retinol Equivalent					
Intake (kCal)	-	-	389.7	-	-
% Adequacy	-	-	75.9	-	-
Thiamin					
Intake (kCal)	0.73	0.74	0.68	1.4	(8.1)*
% Adequacy	70.7	71.8	66.7		
Riboflavin					
Intake (kCal)	0.53	0.58	0.56	9.4 *	(3.4)*
% Adequacy	50.9	56.3	54.4		
Niacin					
Intake (kCal)	15.3	16.4	16.3	7.2	(0.6)
% Adequacy	115.5	119.7	119.9		
Ascorbic Acid					
Intake (kCal)	66.8	61.6	53.6	(7.8)*	(13.0)*
% Adequacy	99.2	91.1	80.0		
Fats					
Intake	28	30	30	7.1 *	-
Carbohydrates					
Intake (kCal)	332	327	313	(1.5)	(4.3)*

\* Statistically significant

Table 9. Dietary score of food intake using recommended DDP.

Food Group	1978		1982		1987	
	% Cal	Score	% Cal	Score	% Cal	Score
Cereals	71.6	35.8	69.8	34.9	69.2	34.6
Roots and Tubers	2.4	1.2	2.3	1.2	1.3	0.7
Animal Products	10.6	26.55	10.8	27.0	11.6	29.0
Added Fat	4.5	4.5	5.2	5.2	5.2	5.2
Nuts and Oilseeds	0.6	0.6	1.3	1.3	1.1	1.1
Pulses and Beans	1.3	1.95	1.3	1.95	1.5	2.25
Sugar	3.8	0.95	4.5	1.13	4.8	1.2
Fruits and Vegetables	4.5	9.0	3.7	7.4	4.3	8.6
Beverage and Seasonings	0.7	0	1.0	0	1.0	0
<b>TOTAL</b>	<b>100.0</b>	<b>80.5</b>	<b>100.0</b>	<b>80.0</b>	<b>100.0</b>	<b>82.6</b>

Table 10. Prevalence of goiter by age, sex and physiologic state. Philippines, 1987.

Population Group	Total Subjects	Total with Goiter	Goiter Classification			
			Grade IA	Grade IB	Grade 2	Grade 3
			Percentage Prevalence			
<b>Males</b>						
7-14 years	2383	0.6	0.4	0.2	0.0	0.0
15-20 years	994	3.1	1.6	1.3	0.1	0.1
21 years & over	4244	1.5	0.8	0.4	0.3	0.0
<b>Females</b>						
7-14 years	2195	4.5	2.5	1.1	0.8	0.1
15-20 years	841	15.2	7.4	5.7	2.0	0.1
21 years & over	3666	10.4	4.6	3.1	2.1	0.6
<b>Pregnant</b>						
< - 20 years	84	26.5	19.5	5.3	1.7	0.0
21-49 years	693	23.4	9.9	8.7	4.3	0.5
<b>Lactating</b>						
< - 20 years	76	24.1	11.9	12.1	0.1	0.0
21-49 years	962	18.1	7.5	7.2	3.0	0.4
<b>ALL</b>	<b>16138</b>	<b>6.9</b>	<b>3.2</b>	<b>2.3</b>	<b>1.2</b>	<b>0.2</b>

**Table 11. Food supply requirement at level of 2600 kcal per capita per day based on recommended DDP.(In thousand metric tons per year).**

<b>Food Group</b>	<b>1995</b>	<b>2000</b>	<b>2010</b>	<b>2020</b>
Cereals	10003.87	11204.49	13833.29	17078.86
Roots and Tubers	2460.77	2756.10	3402.74	4201.09
Animal Products	12203.42	13668.02	16874.81	20833.97
Added Fat	855.42	957.72	1182.42	1459.84
Nuts and Oilseeds	272.30	304.98	376.53	464.87
Pulses and Beans	1607.44	1800.36	2222.76	2744.26
Sugar	738.23	826.83	1020.82	1260.33
Fruits and Vegetables	9770.71	10943.35	13510.87	16680.80
Beverage and Seasonings	2109.23	2362.37	2916.63	3600.93

*\*At a level of 2600 kcal per capita per day*

**Table 12. Food supply requirement at level of 2700 kcal per capita per day based on recommended DDP. (In thousand metric tons per year).**

Food Group	1995	2000	2010	2020
Cereals	10388.64	11635.44	14365.34	17735.74
Roots and Tubers	2555.42	2862.11	3533.61	4362.67
Animal Products	12672.78	14193.71	17523.84	21635.28
Added Fat	887.98	994.55	1227.90	1515.99
Nuts and Oilseeds	282.77	316.71	391.01	482.75
Pulses and Beans	1669.26	1869.60	2308.25	2849.81
Sugar	766.62	858.63	1060.08	1308.80
Fruits and Vegetables	10146.51	11364.24	14030.52	17322.37
Beverage and Seasoning	2190.36	2453.23	3028.81	3739.43

*\*At a level of 2700 kcal per capita per day*

**Table 13. Food supply requirement at level of 2800 kcal per capita per day based on recommended DDP.(In thousand metric tons per year).**

<b>Food Group</b>	<b>1995</b>	<b>2000</b>	<b>2010</b>	<b>2020</b>
Cereals	10773.40	12066.38	14897.39	18392.62
Roots and Tubers	2650.06	2968.11	3664.49	4524.25
Animal Products	13142.14	14719.40	18172.87	22436.59
Added Fat	920.87	1031.39	1273.37	1572.13
Nuts and Oilseeds	293.24	328.44	405.50	500.63
Pulses and Beans	1731.09	1938.85	2393.74	2955.36
Sugar	795.02	890.43	1099.35	1357.28
Fruits and Vegetables	10522.30	11785.14	14550.17	17963.93
Beverage and Seasonings	2271.48	2544.09	3140.99	3877.93

*\*At a level of 2800 kcal per capita per day*

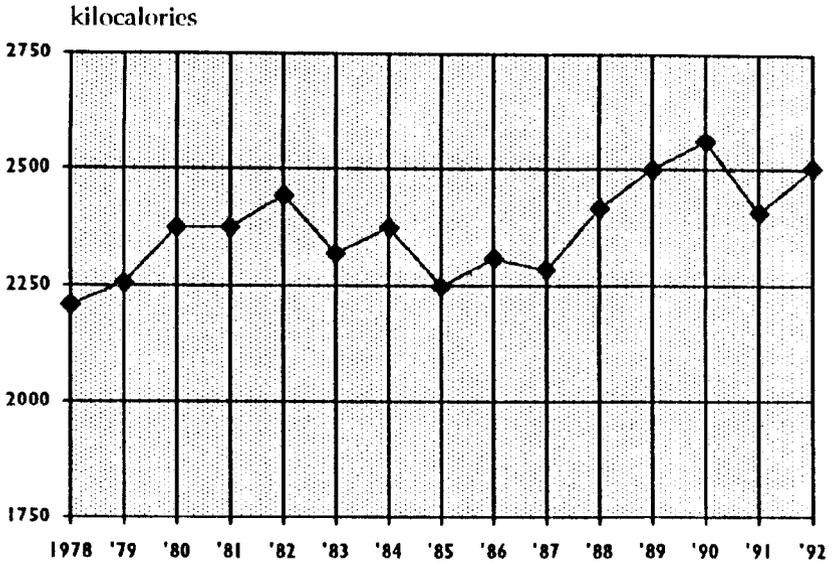


Figure 1. Daily per capita calorie supply, Philippines.

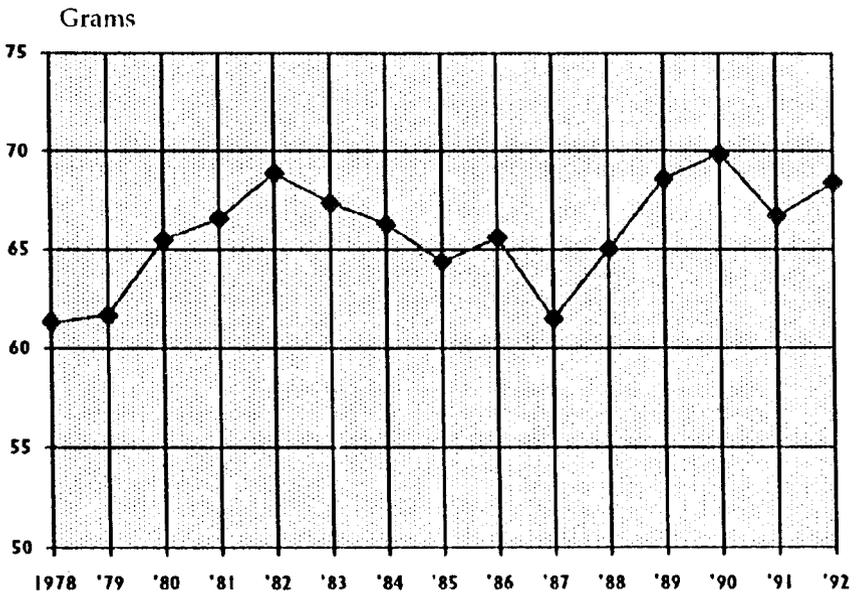


Figure 2. Daily per capita protein supply, Philippines.

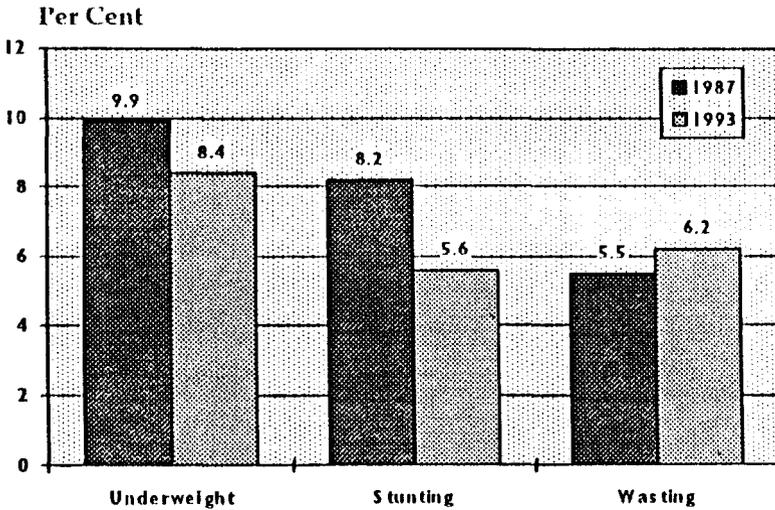


Figure 3. Prevalence of malnutrition among preschool children using various anthropometric indicators, Philippines, 1993.

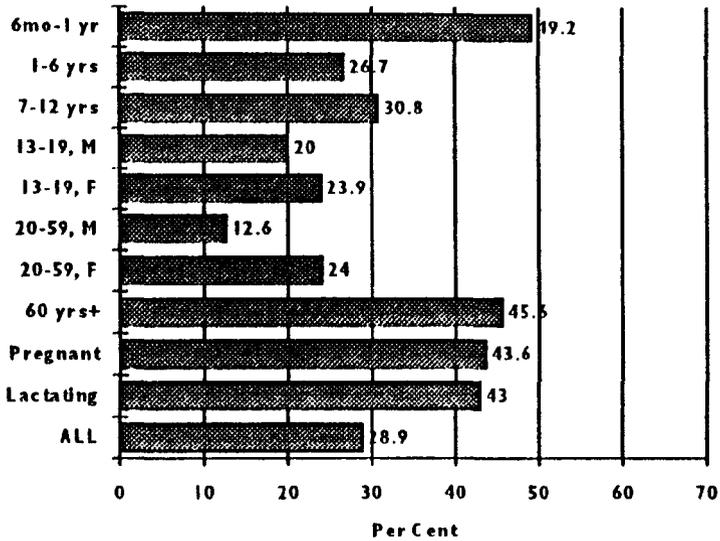


Figure 4. Prevalence of anemia, Philippines, 1993.



**Session 2**  
**Food Processing,**  
**Packaging Equipment**  
**and Machinery**



# **SIMULTANEOUS BIOCONVERSION OF CARBOHYDRATE AND BIODEGRADATION OF CYANIDE IN CASSAVA MEAT TO INCREASE NUTRITIVE VALUE**

*Chay B. Pham, Laura J. Pham and Teresita J. Ramirez  
National Institutes of Biotechnology and Applied Microbiology  
University of the Philippines Los Baños*

## **ABSTRACT**

The major problems associated with cassava tubers are the rapid deterioration of quality after few days of harvest, and its cyanide content. The simultaneous processes for bioconversion of carbohydrate into protein and biodegradation of cyanide were studied using solid state fermentation.

The protein content in the fermented cassava increased to 28-30% (d.w) at the optimized process conditions. The amino acid profile of fermented and raw cassava meat showed an increase in both the essential and non-essential amino acids particularly lysine and methionine. An increase of these amino acids from 12.6 and 3.00 mg/100g in raw cassava meat to 198.51 and 76.17 mg/100g in fermented cassava meat, respectively, were observed.

The results also showed that free and total cyanide concentration of the product greatly decreased after fermentation. Toxicological tests and feeding trials revealed that fermented cassava tubers can be utilized as animal feeds.

## **INTRODUCTION**

The availability of good resources in the developing countries is one of the preoccupying problems at present. Although many regions are deficient in protein, the supply of carbohydrates especially as starch is abundant. Confronted with this situation, it is desirable to develop and diversify agricultural production or look for new sources of protein through biotechnology. Starchy substrates, for instance, could be converted into proteins by the action of microorganisms through solid-state fermentation. The transformations essentially consists of breaking down carbohydrates into utilizable forms which the microorganisms use for growth and metabolism. Organoleptic qualities such as odor and taste are modified during fermentation.

This research was undertaken to develop a low technology fermentation process for the conversion of cassava tubers into a protein-rich product which can be used as animal feed or possibly as human food. At present, the feed requirement of the livestock industry necessitates the importation of protein-acids. When commercialized the bioconversion of starchy materials into protein-rich feed ingredients will definitely cut down the need for importing feeds. This will also boost the livestock industry and maximize utilization of indigenous

materials.

Cassava is known to contain natural toxins, two cyanogenic glucosides, namely, linamarin and lotaustralin (1). The former accounts for 93% of the cyanogenic glucosides and the latter, 7% (2). Cassava is commonly divided into three groups on the basis of the cyanide content in the edible portions of the roots. The sweet or non-toxic group contains 50 mg/kg fresh weight; the intermediate, 50-100 mg/kg; and the bitter or toxic variety, >100 mg/kg (3). Hydrocyanic acid a known potent poison is a product of the reaction of these cyanogenic glucosides.

This work, aims to evaluate the effects of fermentation on the nutritive value and cyanide degradation of cassava tubers.

## MATERIALS AND METHODS

### Materials

Cassava tubers were purchased from Los Banos Public market. These were peeled and cut into approximately 2 mm<sup>3</sup> size. The materials were either fresh or dried in a ventilated air drier at 70°C until moisture content of about 6-7% was reached.

The dried raw materials were milled using Wiley mill to desired particle size.

### Microorganisms

Different fungal strains were screened for bioconversion of cassava tubers. These were obtained from the BIOTECH Microbial Culture Collection Laboratory.

The microorganisms were maintained in Potato Dextrose Agar slants at 4-7°C.

### Media Use

The seed medium consisted of (g/L): glucose, 15; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15; KH<sub>2</sub>PO<sub>4</sub>, 2; K<sub>2</sub>HPO<sub>4</sub>, 2; MgSO<sub>4</sub>, 0.5; NaCl, 1; FeSO<sub>4</sub>, 0.2; and yeast extract, 2. The pH was adjusted to 3.5 using 6M H<sub>3</sub>PO<sub>4</sub>.

The fermentation medium consisted of (g/L): urea, 35; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15; KH<sub>2</sub>PO<sub>4</sub>, 4.5, 3.5; yeast extract, 0.5; and sucrose, 10. The pH was adjusted to 4.5 using 6M H<sub>3</sub>PO<sub>4</sub>.

### Preparation of Inoculum

Spore suspension was prepared from various strains of molds grown on PDA slants aged 3 days. Ten ml of seed medium were added to each culture slants and the spores scraped with an inoculation loop. The spore suspensions were collected in an empty sterile E. flask and vigorously mixed using a vortex mixer. It was used to inoculate the seed medium contained in E. flasks. The seed medium was then shaken for 24-36 hours.

### Solid State Fermentation

The dried and milled substrates contained in polypropylene plastic bags were sterilized at 121°C to 15 min. When cooled, sterile fermentation medium and 24 to 36-hour-old seed medium were added aseptically. For every kg of dried material, 700 ml of fermentation medium and 250 ml of seed medium were used. The substrate was then thoroughly mixed and transferred into trays for fermentation. Batch fermentation process was carried out. The schematic diagram of experimental set-up is shown in Figure 1. It was also used in the optimization of fermentation conditions. It consists of air compressor or air supplier, air flow meter, air filter, humidifier containing saturated  $K_2SO_4$  solution, sparger, stationary tray bioreactor, trays and 2N NaOH solution tank.

A scale bioreactor was also used to produce larger quantities of product. Its system is similar with the experimental set-up shown in Figure 1. However, each tray contained in this bioreactor could accommodate 3-4 kgs of materials.

### Downstream Processing

After 3-4 days fermentation or when the mold has grown into the mycelial stage, the products were dried in an air-ventilated drier at 70°C for 24 h or until the moisture content of the product reached 6-7%.

The dried products were then ground using a Wiley mill and stored in plastic bags at room temperature.

### Analytical Methods

The protein-enriched products and the raw materials were analyzed to determine changes brought about by fermentation.

1. Proximate analysis was performed following the AOAC method (4).
2. pH was determined by mixing one gram sample with 20 ml distilled water, pH 7.0 and mixed for 20 minutes using magnetic stirrer. The pH of the solution was measured using Orion Digital pH meter.

3. Soluble protein was determined with Folin-phenol reagent following Lowry method (5).
4. Crude protein was determined by a micro-Kjeldahl procedure on duplicate samples, and the factor 6.25 was used to calculate crude protein.
5. Amino acid profile was determined by sending samples to the University of Korea for analysis.

### **Toxicology Analysis**

The following methods were used in evaluating the toxicity of the products:

1. Thin layer chromatography was used to assay mycotoxins in fermented products. The methods of AOAC (1984) was used with some modifications in the clean-up and developing stages (6,7).

Extraction of mycotoxins was either done in a 22 by 100 mm chromatography tube containing sodium sulfate, silica gel and chloroform, or in separatory funnel with chloroform mixture.

Preliminary thin layer chromatography was done in developing glass tanks containing developing solutions specific for each mycotoxin being examined. The developed plates were then viewed under longwave and shortwave UV lamp in a Chromato-VEU cabinet. The characteristics of fluorescent spots and Rf values were noted.

2. Chemical analysis was performed to determine the total and free cyanide in the fermented samples. The alkaline picrate method (8) was followed. Total and free cyanide were determined by adding an aliquot of 0.1 ml each of the sample extract into stoppered test tubes containing 0.4 ml of 0.2 M phosphate buffer (pH 7.0). Aliquot of 0.1 ml of linamarase was then added to each test tube containing samples for total cyanide determination. Aliquot of 0.1 ml of 0.11 phosphate buffer (pH 6.0) was added to test tubes containing samples for free cyanide. The tubes were then incubated at 30°C for 15 min, then the reaction was stopped by adding 0.6 ml of 0.2M NaOH.

3. The chicken embryo technique (4) was also followed in evaluating the toxicity. The study was done at the Bureau of Animal Industry poultry farm in Alabang. Both the water - and oil-soluble extracts were evaluated for toxicity.

4. Using Swiss White mice, skin toxicity test was performed (9). After dosing, the mice were observed frequently for 48 hrs and then sacrificed by diethyl ether inhalation. The degree of toxicity was recorded as follows: (D),

death; (+), definite skin symptoms in the shaved are; (-), no positive evidence of toxic effect.

### **Feeding Trials**

Different protein-enriched materials were used in the feeding trials. In some cases, one or two materials were only evaluated.

#### **1. Mice Feeding**

Swiss White breed mice with an average weight of about 18 gm were used in the study. They were housed in plastic cages with the males separated from the females. They were provided with water and food ad libitum. Wood shavings were provided as bedding materials.

To allow individual identification, each mouse was color-coded with permanent non-toxic markers. The control group received commercial diet in the form of pellets whereas formulated ration of fermented material were given to the other group. Care and maintenance of the cages were done by changing the bedding materials every 4 days. There was first a one week adjustment period before the start of the study. Daily gain in weight was calculated.

At the end of the study, the mice were sacrificed. The internal organs were kept in bottles containing 10% buffered formalin solution. The hispathological examination was done at the College of Veterinary Medicine, UPLB.

#### **2. Fish Feeding**

Aerated glass aquaria measuring 30 x 75 x 30 cm were used in the study. Tilapia fingerlings weighing 6-7 grams were stocked at 20 fishes per aquarium. Each aquarium was subjected to different feed rations containing 32-33% protein, this being the required level for tilapia. The protein-enriched products were the protein sources in the experimental rations which replaced soybean and fish meal in the control diet.

Vitamin-mineral mixture and codliver oil were premixed with a small quantity of the feedstuffs and was mixed manually. The mixed feeds were placed in a plastic container and stored in a refrigerator.

The fish wer fed twice daily at a rate of 5% fish body weight per day for 63 days. Daily cleaning of aquaria was also done. The body weight gain of the fish was monitored.

#### **3. Broiler Feeding**

The chicks were randomly allotted to different treatments following a randomly complete block design. Different levels of substitution of protein-enriched cassava were used as treatments.

On the first week, the chicks were fed booster diets, and on day 8, they were distributed at random. They were fed with their respective starter ration from day 8 to day 35 and with finisher ration from day 36 to day 49. Feeds and water were given ad libitum. The growth performance of the chicks was monitored throughout the 49-day feeding period.

## RESULTS AND DISCUSSION

### Screening for Protein-Enrichment of Cassava Tubers

A total of 32 fungal strains were screened for bioprocessing of cassava tubers. Table 1 shows that fermentation effected significant increases in the total protein content of cassava. From an initial crude protein level of about 1-2%, the fermented cassava contained about 12-25% protein after 72 h fermentation depending in the fungal strain used. *A niger* BIOT 3104 was selected and used in succeeding experiments for process optimization studies.

The increase in the protein components may be attributed to the contribution made by the microorganisms. As expected of a bioconversion process, an increase in the protein content is accompanied by a decrease in the carbohydrate content. The microorganisms utilize the carbohydrate, the non-protein nitrogen sources and minerals present initially in the substrates for their growth and metabolism. The protein-enriched product is a mixture of the microbial biomass and the residual raw materials. This type of biotechnological processing does not involve any separation step.

The conditions for protein production were optimized using the stationery tray bioreactor (STB). As fermentation progressed, changes in the total protein content, starch content and free reducing sugars were observed. There was an increase in the free reducing sugar content during the active growth phase (72 h). This pattern has been previously observed (10) in the case of *A. niger* on cassava meal which was attributed to a starch hydrolysis rate faster than glucose utilization. This accumulation of free sugar remained small compared to the overall consumption of equivalent hydrolyzed sugars from starch and stops increasing after growth has ceased. This maybe attributed to the decrease of amyolytic activity of the mold upon aging. As expected of a bioconversion process, the protein content increased while the starch concentration decreased (Figure 2). At the maximum of protein curve, there is an important sugar consumption which can be attributed to a maintenance equivalent to a total oxidation of the substrate.

The designed STB consisting of 4 perforated trays lined with aluminum foil, on which 0.25 cm-deep layer of fresh substrate with the inoculum was spread to ferment at an operating temperature of 30°C and air flowrate of 0.257 L/min. is very satisfactory since the results obtained gave an average of 25-27% total protein content after 72 h of fermentation. The protein production obtained from the different tray levels approximatre each other (Figure 3). The results suggest

that tray location has no influence on the protein production. Beyond 72 h a decrease in protein was observed which maybe due to sporulation.

These results corroborate with the findings of several researchers (10, 11) on a number of starchy raw materials which could be used as sources for protein enrichment. From cassava, banana, potato and starchy wastes, fermented products with a 16 to 20% protein content and 25 to 35% residual sugars were obtained which are considered adequate for animal feed. Lower value for protein of about 11% dry basis were also obtained by other researchers (12).

### **Amino Acid Profile of Raw and Fermented Substrates**

The amino acid profile usually serves as the basis for selecting protein-rich foods which contain the essential amino acids needed for human and animal feeding.

Comparative amino acid profile of initial (raw) and fermented substrates is presented in Table 2. The data reveal that the fermented products are good sources of lysine, methionine, phenylalanine and leucine. There was significant increases in the amount of both essential and non-essential amino acids in the fermented products. In most cases, the amino acid content after fermentation increased more than two-folds.

### **Toxicological Evaluation of Fermented Products**

#### **1. Thin Layer Chromatography**

The absence of toxic metabolites, the safety of the fermented products have to be established before any recommendation for use as feed or food can be made.

The preliminary results from TLC as presented in Table 3 showed that mycotoxins were not present in the fermented products. These samples tested were fermented and dried right away. Samples used for mycotoxin analysis and for the other toxicity tests were all based on newly prepared protein-enriched products.

Some of the cassava from Matling Corp., used in this study were of the bitter variety. The cyanide content of the unfermented materials were far above the level of 100 mg/kg fresh weight (Table 4). The bitter variety is usually used for starch production and not for food or feed. However, fermentation significantly decreased the amount of cyanide present, thus rendering the fermented product safe for animal consumption.

This biotechnology process, the cassava was subjected to several treatments; washing, drying, grinding, sterilization and fermentation which greatly lowered

the cyanide content. The fermentation process is the major detoxification process. This is attributed to the spontaneous hydrolysis of the constituent cyanogenic glycosides, principally linamarin, by the organic acids produced during starch degradation by the fermentative action of the microorganisms. It has been shown that detoxification is due mainly to the action of linamarase (linamarin B-D-glucoside glucohydrolase) on the cyanogenic glucosides of fermenting cassava (8).

### **Chicken Embryo Test**

The chicken embryos are known to be sensitive to aflatoxins. The lowest  $LD_{50}$  value of 0.02 ug/embryo has been reported (13). The results of chicken bioassays showed that the treated embryos showed comparative performance with the control (Table 5).

### **Mice Toxicity Test**

When extracts of the microorganisms were administered to mice either intraperitoneally or orally, there were differences in weight gain of the animals. The treated mice responded better when compared with the untreated group. Those that received extracts orally also had higher weight gain than those given doses intraperitoneally, this being the case for both the male and female mice (Table 6).

### **Feeding Trials**

Table 7 shows the weight gain of mice fed control diet and protein-enriched cassava for 4 weeks. The male and female groups, exhibited differences in the ADG, however in the treated group than the control.

The increase in mean fish biomass production and overall % body weight gain of fish given either fermented cassava meal or unfermented point is presented in Table 8. After 63-days feeding period biomass production and weight gain were higher in fish group given fermented cassava. The value in total biomass production and over-all % body weight are in general agreement with values of growth rate. The growth rate of fish given fermented cassava was 0.18 g/day as compared with 0.16% g/day in the control group.

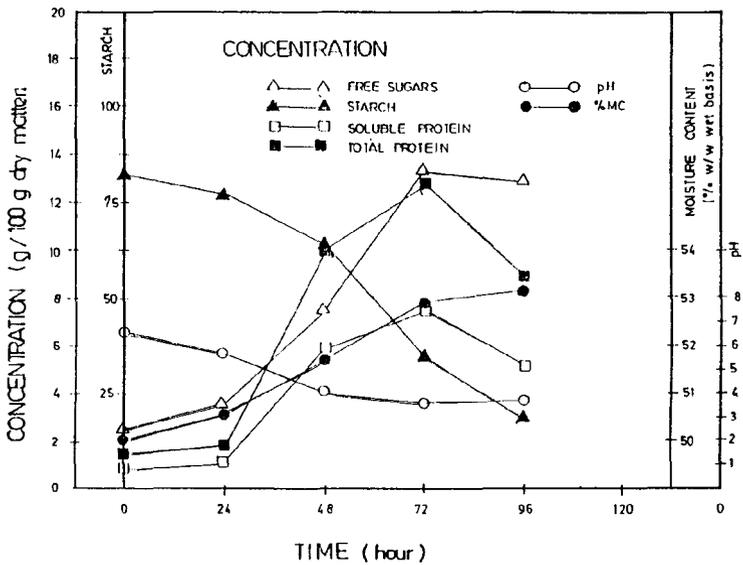
**LITERATURE CITED:**

1. Coursey, D.G., 1973. Cassava as Food: Toxicity and Technology. IDRC. Canada.
2. Seerly L.M. and Rodriguez- Amaya D. 1988. Screening and quantitation of ochratoxin A in corn, peanut, bean rice and cassava. Journal of Association of Official Analytical Chemists. 68 (6): 1128 - 1130.
3. Fukuba, H. and E.M. T. Mendoza. 1984. Determination of cyanide in cassava. Tropical Root Crops: Postharvest Physiology and Processing. (Japan Scientific Societies Press), pp. 171-181.
4. A.O.A.C. 1980. 1980. Official Methods of Analysis of the Association of Official Analytical Chemists. In: Horowitz, W. (ed) 13th edition, Washington, D.C.
5. Lowry, O.H., N.S. Resebrough, A. Lewisfarr and R.J. Randall. 1951. Protein measurement of the folin-phenol reagent. Journal of Biological Chemistry. 193-265-275.
6. Soares, L.M. and Rodriguez- Amaya. D. 1988. Screening and quantitation of ochratoxin A in corn, peanut, bean rice and cassava. Journal of Association of Official Analytical Chemists 68 (6)1128-1130
7. Soares, L.M. and Rodriguez- Amaya. D. 1988. Survey of aflatoxin, ochratoxin A, zearalenone and sterigmatocystin in some Brazilian foods by using multi-toxin thin-layer chromatographic method. Journal of Association of Official Analytical Chemists. 72 (1): 22-26.
8. Ikediobi, G.O., C. Onijia and C.E. Eliwah. 1980, A rapid and inexpensive assay for total cyanide in cassava. Journal of Agricultural Biological Chemistry. 44 (12): 2803-2809.
9. Abbas, H.K., C.J. Morocha and W.T. Shier. 1984. Mycotoxin produced from fungi isolated from foodstuffs and soil: comparison of toxicity in fibroblasts and rat feeding tests. Journal of Applied and Environmental Microbiology. 48:654-661.
10. Raimbult, M.F. Deschamps, F. Mayer and J.C. Senez, 1977. Proceedings of the 5th International Conference on Global Impacts of Applied Microbiology. November 21-26, 1977. Bangkok, Thailand.

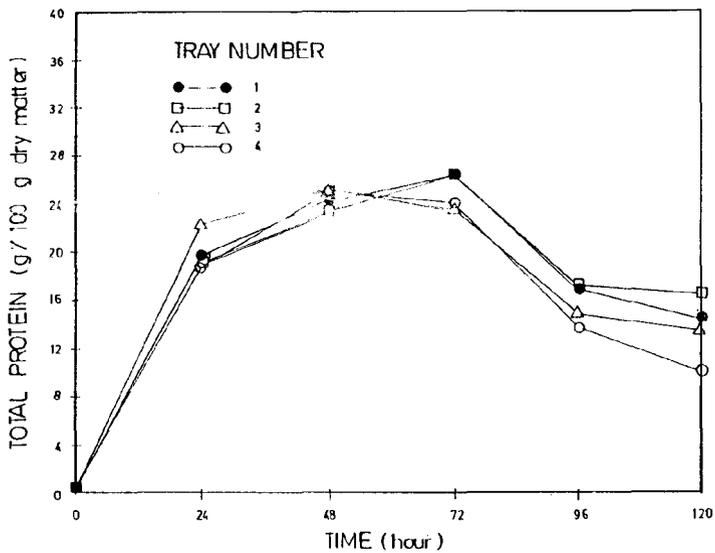
11. Baldensperger, J., J. Le Mer, L. Hannibal and P.J. Quinto. 1985. Solid state fermentation of banana wastes. Biotechnology Letters. 7(10):743-748.
12. Daubresse, P., A. Ntibashirwa, A. Gheysen and J.A. Meyer. 1986. A process for protein-enrichment of cassava by solid substrate fermentation in rural conditions. Biotechnology and Bioengineering. 29:962-9968.
13. Bilgrami, K.S. and R.S. Mitra. 1979. Aflatoxin production by *Aspergillus flavus* in storage and standing maize crops. Advancing Frontiers of Mycology and Plant Pathology. pp 67-68.

**Table 1.** Protein enrichment with different microorganisms by solid-state fermentation.(DM= Dry matter).

Strain	Soluble Protein	Total Protein
	(g/100g DM)	
<i>A. awamori</i> 3112	6.83	11.69
<i>A. niger</i> 3105	3.84	10.68
<i>A. niger</i> 3104	6.57	11.25
<i>A. niger</i> 3080	7.30	9.02
<i>A. japonicus</i> 3093	5.26	10.10
<i>T. reesei</i> 3101	1.99	11.79
<i>T. harzianum</i> 3001	1.40	7.32
<i>T. viride</i> 3110	1.97	11.64
<i>T. harzianum</i> 36	1.68	9.44



**Figure 2.** Changes in pH, moisture, protein and carbohydrate yield during SSF of cassava at 30°C, 0.257 L/min air flowrate and 50% initial moisture content.



**Figure 3.** Effect of tray location in STB on protein yield at 30°C, 0.257 L/min air flowrate, 50% initial moisture content, 0.25cm substrate layer thickness and pH 2.0.

**Table 2. Amino acids (mg/g N) composition of raw and fermented cassava tuber.**

Amino acids	Amount (mg/g N)		
	Raw		Fermented
	I <sup>a</sup>	II <sup>b</sup>	
Alanine	-	-	814
Arginine	380	672	688
Aspartic acid	1	1	974
Cysteine	64	20	0
Glutamic acid	-	-	2320
Glycine	0	0	612
Histidine	123	74	450
Isoleucine	209	128	604
Leucine	298	195	902
Lysine	392	258	840
Methionine	97	107	320
Phenylalanine	182	138	742
Proline	-	-	902
Serine	-	-	484
Threonine	210	20	484
Tyrosine	64	81	426
Valine	248	175	944

Sources: <sup>a</sup>Food Composition Table. 1990. 6th Ed. Handbook. FNRI, DOST.

<sup>b</sup>Nutrient Composition of some Philippine Feed Stuffs. 1979.

**Table 3. Results of mycotoxin analysis using TLC of selected fermented samples following the modified procedure of Soares and Rodrigues-Amaya (1989).**

Sample	Result
Cassava, unfermented	Aflatoxin negative
Cassava, 0 day	Aflatoxin negative
Cassava, fermented for 4 days	Aflatoxin negative Citrinin negative Zearalenone negative Ochratoxin negative
Cassava pulp, unfermented	Aflatoxin negative
Cassava pulp, fermented	Aflatoxin negative

**Table 4. Cyanide content of unfermented and fermented cassava tubers.**

Sample	Cyanide Concentration (mg/kg sample)	
	Free	Total
Raw cassava pulp	242.16	1210.81
Fermented cassava pulp	19.18	95.45
Raw cassava meat	561.52	1553.05
Fermented cassava meat	43.18	117.89

**Table 5. Results of bioassay toxicity test on chicken embryo with oil-and water-extracts of various fungal isolates used in protein-upgrading of different agricultural materials.**

Isolate	Kind Extract	Ave No. Dead First	Embryo Second	No. Eggs Hatched	No. Weak & Condemned Chicks
Control	Water	2	3	8	2
	Oil	2	2	10	1
<i>A. niger</i>	Water	1	1	13	2
	Oil	2	4	7	2
<i>T. harzi-anum</i>	Water	1	2	10	2
	Oil	2	1	10	2
<i>T. viride</i>	Water	1	0	13	5
	Oil	4	1	9	5

**Table 6. Results of bioassay tests on Swiss White breed mice using fungal extracts.**

Fungal Extract	Cumulative % Body Weight Gain			
	Oral Dose		Intraperitoneal Dose	
	Male	Female	Male	Female
<i>Aspergillus niger</i>	3.50	9.42	7.23	2.96
<i>Trichoderma harzianum</i>	-0.47	11.93	11.68	8.94
<i>Trichoderma viride</i>	0	10.73	-0.71	4.81
Control	8.60	10.58	2.76	7.82

**Table 7. Comparison of the average daily gain of male and female Swiss White breed mice given protein-enriched diet and control diet.**

Week No.	Treatment			
	Protein-enriched Cassava Diet		Control Diet	
	Male	Female	Male	Female
0	0.000	0.000	0.000	0.000
4	(0.355)	(0.125)	0.185	0.190
8	0.645	0.180	0.142	(0.090)
12	0.300	0.150	0.62	0.150
16	0.115	(0.085)	0.075	0.135
20	(0.035)	0.100	0.145	0.015
24	0.930	0.120	0.500	0.160
28	(0.330)	.440	(0.092)	0.095
32	1.000	0.145	0.533	0.150
Average	0.284	0.119	0.225	0.104

**Table 8. Comparative performance of tilapia fed with control diet and protein-enriched cassava meat and cassava pulp.**

	T r e a t m e n t		
	Control Diet	Protein-Enriched Cassava Meat	Protein-Enriched Cassava Pulp
Initial Weight (g)	6.41	6.70	6.74
Total fish biomass (g)	128.2	134.0	134.8
Final Weight (g)	17.84	19.02	22.67
Total fish biomass (g)	231.9	266.3	294.70
Gain in weight (g)	11.43	12.32	15.93
Growth rate (g/day)	0.16	0.18	0.23
Overall gain in weight (%)	178.31	183.88	236.35
Survival (%)	65.00	70.00	65.00

# PRODUCTION OF DIETARY FIBER FROM BAGASSE

*Rosita P. Ebron, Rosemarie S. Gumera,  
Rosalina B. Tan & Angelina, M. Lojo  
Sugar Regulatory Administration, Philippines*

## ABSTRACT

Dietary fiber was isolated from whole bagasse and bagasse pith by combination of processes involving prehydrolysis with water, delignification with caustic soda and multi-stage bleaching with calcium hypochlorite.

Based on economic evaluation in terms of chemicals used, isolation of dietary fiber from bagasse pith costs less than equivalent isolation from whole bagasse.

The process recovered 19-33% dietary fiber from whole bagasse and 29-38% dietary fiber from bagasse pith. The dietary fiber isolated was composed of 55-56% alphacellulose, 43-44% hemicellulose, 0.4-0.6% lignin and minimal amount of chemical residues.

## INTRODUCTION

Dietary fiber is one of the most exciting and controversial topic in human nutrition. It continues to be one of interest to nutritionists since it has been accepted that a number of diseases prevalent in rich societies are caused by lack of these substances in modern diet. The call for an increased consumption of dietary fiber is certainly welcomed. In 1977, a workshop on dietary fiber and disease, organized by the European Economic Community and the International Agency for Research on Cancer was held in Lyon, France (2). Nutritional and physiological effects of dietary fiber upon human became a matter of renewed concern and research activity.

A dietary fiber from the husks or outer parts of cereal grain was developed by Lennart Holmgren of Sweden. He found out that fiber obtained from husks of cereal grain contains 70% fiber (3).

Dietary fiber was defined by Hipsley (4) to include lignin, cellulose and hemicellulose, and has been broadened by some to include soluble substances such as pectins, gums and mucilages. This current broad definition acknowledges the significance of fiber as a chemical and physiological component of the diet as compared with the static definition of crude fiber, "the residue of plant food left after extraction with solvent, dilute acid and dilute alkali".

By 1981, at the 95th AOAC Annual Meeting, (4) more than 100 responses had been received expressing interest in dietary fiber and suggesting definitions of dietary fiber and a preferred method of analysis. Most respondents preferred the definition of Trowell, "Dietary Fiber consists of remnants of the plant cells

resistant to hydrolysis by the alimentary enzymes of man". This definition was later modified by Trowell et al. and Van Soest and McWueen to include hemicelluloses, celluloses, lignins, nondigestible oligasaccharides, pectins, gums and waxes.

It is generally accepted that dietary fiber is not inert, on the contrary, it can interact considerably with various components of the diet. Burkitt and Trowell (1974) (2) reviewed experimental and epidemiological data and suggested that low intake of dietary fiber in Western countries might contribute to the occurrence of diverticulitis and carcinoma of the colon, cardiovascular disease, diabetes and obesity.

Dietary fiber may have a protective effect against gastrointestinal cancer. It may protect the intestine from carcinogenic agents by enhancing intestinal transit time and / or directly binding the cancer causing chemicals thereby preventing absorption and enhancing excretion (5). Dietary fiber has the ability to absorb organic molecule such as bile acids. Absorption of bile acids would result in two changes in cholesterol metabolism which would cause a loss of cholesterol from the body. First, increased bile acid excretion would necessitate increased synthesis of bile acids from cholesterol.

Second, the unavailability of bile salts in the small intestine for micelle formation would also inhibit cholesterol absorption (2).

Diets rich in fiber produce bulky, soft stools which traverse the gut rapidly, while diet low in fiber (refined diet) produce small, hard stools. An inverse relationship between stool weight and transit time has been reported by many researchers (2).

Dietary fiber from cane bagasse is considered safe for human consumption. The cane stalk itself has been traditionally chewed with no reported side effect. No toxic component was found in cane bagasse nor in the juice extracts containing some of the tissues residues. The leaves are used for feeding cattles.

Isolated dietary fiber is completely a new product with prospective uses as flour supplement for baked goods and noodle, and as fillers for dietary pills.

## MATERIALS AND METHODS

### Preparation of Cane Bagasse

Whole bagasse was first air-dried and oven-dried until bone dry then ground in a Wiley/Laboratory Mill through a 2-mm screen. Pith was prepared by removing the rind manually from whole bagasse, dried and ground as in whole bagasse. Whole bagasse and bagasse pith were analyzed for moisture, alphacellulose, hemicellulose and lignin content.

### Isolation of Dietary Fiber from Whole Bagasse

Seven experimental runs using 100-g sample of ground whole bagasse were evaluated. In the digestion stage, each sample was treated with varying concentrations of caustic soda (sodium hydroxide), varying liquor ratios and reaction conditions of time, temperature and pressure. In the bleaching stage, each sample was subjected to a four-stage bleaching operation and treated with varying ratios of caustic soda and calcium hypochloride and various reaction conditions. Pulp samples were washed and strained after each treatment. The product was dried at 80°C for 8 hours and finally ground in a Willey Mill through a 0.5mm screen. The yield and potassium permanganate number were determined for each process. Alphacellulose, hemicellulose, lignin, moisture content, density, pH and water-holding capacity of the final product were determined and analyzed.

The chemical residues retained by dietary fiber were analyzed at the Bureau of Food and Drug Laboratory.

### RESULTS AND DISCUSSION

The proximate chemical analysis of whole bagasse and bagasse pith is shown in Table 1.

Based on the results of preliminary experimental runs, dietary fiber can be isolated from whole bagasse and bagasse pith by a combination of digestion and bleaching processes using two delignifying agents, caustic soda and calcium hypochlorite.

Considering the potassium permanganate number (6), percent yield and economic evaluation in terms of chemicals used, the optimum process for the isolation of dietary fiber from whole bagasse is run #5 of Table 2. In this process, bagasse was prehydrolyzed with 10 parts water to 1 part bagasse, by weight, at 15 psig for one hour. The pulp was digested with 2.0% caustic soda solution, liquor ratio of 2:1 at 15 psig for one hour. The bleaching process was subdivided into four stages. In the first stage, calcium hypochlorite alone was used as the bleaching agent. The succeeding three stages was a combination of caustic soda and calcium hypochlorite. Multiple stage bleaching is essential in order to obtain a soft dietary fiber. Concentration of caustic soda and calcium hypochlorite was controlled so that the essential components of the fiber would not be destroyed chemically.

Isolation of dietary fiber from bagasse pith was found to be optimum using the process presented in run #5 of Table 3. The pith was prehydrolyzed with 12 parts water to 1 part bagasse, by weight, at 15 psig for one hour. It was digested with 2.0% sodium hydroxide solution, liquor ratio of 2:1 at psig for one hour. Three-stage bleaching process was found to be sufficient in reducing the potassium permanganate number of the pulp to an acceptable whiteness. Isolation of dietary fiber from bagasse pith requires less chemicals than that needed to isolate dietary fiber from whole bagasse. Fibers from whole bagasse are composed of hard or sclerenchymatous fibers with thick-walled elongated cells while bagasse pith is composed of parenchymatous materials which is softer and easier to digest.

Cost estimate of chemicals used for the isolation of dietary fiber from whole bagasse is presented in Table 4. The cost of chemicals per kilogram dietary fiber from whole bagasse based on the optimum process is P32.58.

Cost estimate of chemicals for the production of dietary fiber from bagasse pith is shown in Table 5. Based on the optimum process, the cost of chemicals per kilogram dietary fiber is P27.50.

Analysis of the essential components, physical properties and chemical residues is shown in Table 6 and 7. Water-holding capacity was analyzed using two methods; AOAC method for peat (1) and method by Mc Connel et al (1974). pH of a 2.0% solution of dietary fiber is just within the neutral range, 8.4 for dietary fiber from whole bagasse and 8.2 for dietary fiber from bagasse pith. Percent purity of dietary fiber isolated from whole bagasse is 99.6% and the percent purity of dietary fiber derived from bagasse pith is 99.4%

Minimal amount of sodium and chloride ions were present in the dietary fiber. The presence of a small quantity of calcium is not hazardous to health, it aids in the ion-exchange capability of dietary fiber.

A process flowsheet for the production of dietary fiber from whole bagasse is shown in Table 8.

The development of dietary fiber from cane bagasse could be a new product which is essential to the human diet. Further studies on improved technology and quality of dietary fiber has to be undertaken. This is a preliminary study on the production of dietary fiber from bagasse and incorporation of the fiber in food products had been initiated. Diet cookies enriched with dietary fiber from bagasse was launched during Science and Technology Fair at the Philippine Trade and Training Center last July 1993.

#### REFERENCES:

1. Association of Official Analytical Chemists. 1984. 14th Edition. Official Methodss of Analysis. p. 32.
2. Inglett, G. & S. I. Falkenag. 1979. Dietary Fibers: Chemistry and Nutrition. pp. 49-281.
3. Holmgren, L. S. B. of Angelholm, Sweden. 1986. Dietary Fiber Product Philippine Patent No.19672.
4. Prosky et al. 1984. Vol. 67, No. 6. Vitamins and Other Nutrients. Journal of the Association of Official Analytical Chemists. p. 104.
5. Spiller, G. A. Handbook of Dietary Fiber in Human Nutrition. pp. 3-18.
6. Technical Association of Pulp & Paper Industry. T 214 su-71. T 203 os-74. T 222 os-74.

**Table 1. Proximate analysis of whole bagasse and bagasse pith (Dry basis).**

<b>% Composition</b>	<b>Whole bagasse</b>	<b>Bagasse Pith</b>
Moisture	10.2	3.3
Ash	2.3	2.6
Lignin	22.3	22.5
Hemicellulose	31.8	33.2
Alphacellulose	34.9	34.9

**Table 2. Potassium permanganate number and percent yield of dietary fiber from experimental runs on whole bagasse.**

Run No.	Digestion	Bleaching Stage				KMnO <sub>4</sub> No	Percent Yield
		1	2	3	4		
1	6:1, LR* 3.0 % B 1 hr, 15 psig	5:1, 1.0 % A** 1 hr, RT****	2:1, 1.0 % B*** 2 hr, 15 psig 2:1, 2.5 % A 1 hr, RT	2:1, 1.0 % B 2 hr, 15 psig 2:1, 1.5 % A 1 hr, RT	2:1, 1.0 % B 2 hr, RT 2:1, 1.0 % A 1 hr, RT	0	19
2	8:1 1.0 % B 2 hr, 15 psig	4:1, 1.0 % A 2 hr, RT	4:1, 1.25 % B 2 hr, 15 psig 2:1, 2.0 % A 1 hr, RT	2:1, 1.0 % B 1 hr, 15 psig 2:1, 0.5 % A 1 hr, RT	2:1, 0.5 % B 2 hr, RT 2:1, 0.5 % A 1 hr, RT	3.0	20
3	6:1 1.33 % B 2 hr, 15 psig	4:1, 1.0 % A 2 hr, RT	3:1, 1.0 % B 2 hr, 15 psig 2:1, 2.0 % A 1 hr, RT	2:1, 1.0 % B 1 hr, 15 psig 2:1, 0.5 % A 1 hr, RT	2:1, 0.5 % B 2 hr, RT 2:1, 0.5 % A 1 hr, RT	4.0	22
4	PH****, 6:1 1 hr, 15 psig 3:1, 2.0 % B 2 hr, 15 psig	4:1, 1.0 % A 1 hr, RT	2:1, 2.0 % B 2 hr, 15 psig 2:1, 2.0 % A 1 hr, RT	2:1, 1.0 % B 1 hr, 15 psig 2:1, 1.0 % A 1 hr, RT	2:1, 0.5 % B 1 hr, RT 2:1, 0.5 % A 1 hr, RT	2.0	21

Continuation of Table 2.

5	PH, 10:1 1 hr, 15 psig 2:1, 2.0 % B 1 hr, 15 psig	2:1, 1.5 % A 1 hr, RT	2:1, 1.5 % B 1 hr, 15 psig 2:1, 1.25 % A 1 hr, RT	2:1, 1.0 % B 1 hr, 15 psig 2:1, 1.0 % A 1 hr, RT	2:1, 1.0 % B 1 hr, RT 2:1, 1.0 % A 1 hr, RT	2.3	33
6	PH, 10:1 1 hr, 15 psig 2:1, 2.5 % B 1 hr, 15 psig	2:1, 1.5 % A 1 hr, RT	2:1, 1.5 % B 1 hr, 15 psig 2:1, 1.25 % A 1 hr, RT	2:1, 1.0 % B 1 hr, RT 2:1, 1.0 % A 1 hr, RT	2:1, 0.75 % B 1 hr, RT 2:1, 1.0 % A 1 hr, RT	1.9	28
7	PH, 10:1 1 hr, 15 psig 2:1, 3.0 % B 1 hr, 15 psig	2:1, 1.5 % A 1 hr, RT	2:1, 1.5 % B 1 hr, 15 psig 2:1, 1.25 % A 1 hr, RT	2:1, 1.0 % B 1 hr, 15 psig 2:1, 1.0 % A 1 hr, RT	2:1, 0.5 % 1 hr, RT 2:1, 1.0 % A 1 hr, RT	2.2	32

\* Liquor Ratio, ml liquor per gram dry bagasse

\*\* Ca(OCl)<sub>2</sub>

\*\*\* NaOH

\*\*\*\* Room temperature

\*\*\*\*\* Prehydrolysis; ml water per gram bagasse

**Table 3. Potassium permanganate number and dietary fiber yield of experimental runs on the digestion of bagasse pith.**

Run No.	Digestion	Bleaching Stage			KMnO <sub>4</sub> No	Percent Yield
		1	2	3		
1	10:1, LR*, 2.0 % B** 1 hr psig	8:1, 1.0 % A*** 1 hr, RT	4:1, 1.0 % B 15 min. 15 psig 4:1, 1.0 % A 1hr, RT		0	38
2	10:1, 1.0 % B 1 hr, RT ****	10:1, 1.0 % A 1 hr, RT	5:1, 0.25 % B 1 hr, RT 5:1, 0.25 % A 1 hr, RT		0	32
3	PH *****, 12:1 1 hr, 15 psig 2:1, 2.5 % B 1 hr, 15 psig	2:1, 2.0 % A 1 hr, RT	2:1, 2.0 % B 1 hr, 15 psig 2:1, 1.0 % A 1 hr, RT	2:1, 1.5 % B 1 hr, 15 psig 2:1, 1.0 % A 1 hr, RT	2.8	29
4	PH, 12:1 1 hr, 15 psig 2:1, 2.0 % B 1 hr, 15 psig	12:1, 2.0 % A 1 hr, RT	2:1, 1.5 % B 1 hr, 15 psig 2:1, 1.0 % A 1 hr, RT	2:1, 1.0 % B 1 hr, 15 psig 2:1, 1.0 % A 1 hr, RT	4.4	30
5	PH, 12:1 1 hr, 15 psig 2:1, 2.0 % B 1 hr, 15 psig	2:1, 2.0 % A 1 hr, RT	2:1, 1.0 % B 1 hr, 15 psig 2:1, 1.0 % A 1 hr, RT	2:1, 1.0 % B 1 hr, 15 psig 2:1, 1.0 % A 1 hr, RT	3.0	32

\* Liquor Ratio; ml liquor per gram dry pith

\*\* NaOH

\*\*\* Ca (OCl)<sub>2</sub>

\*\*\*\* Room temperature

\*\*\*\*\* Prehydrolysis; ml water per gram dry pith

Table 4. Cost estimate of chemicals used in the isolation of dietary fiber from whole bagasse.

Run No.	% Yield *	Kg Bagasse per kg DF**	Total % NaOH g NaOH	Kg NaOH per kg DF	Total % Ca(OCl) <sub>2</sub> g Ca(OCl) <sub>2</sub>	Kg Ca(OCl) <sub>2</sub> per kg DF	Cost of NaOH (P)	Cost of Ca(OCl) <sub>2</sub> (P)	Total Cost/kg DF (P)
			----- 100 g bagasse		----- 100 g bagasse				
1	19	5.263	24.0	1.263	15.0	0.790	25.26	71.10	96.36
2	20	5.000	16.0	0.800	10.0	0.500	16.00	45.00	61.00
3	22	4.545	14.0	0.636	10.0	0.455	12.72	40.95	53.67
4	21	4.762	13.0	0.619	11.0	0.524	12.38	47.16	59.54
5	33	3.030	11.0	0.333	9.5	0.288	6.66	25.92	32.58
6	28	3.571	11.5	0.411	9.5	0.339	8.22	30.51	38.73
7	32	3.125	12.0	0.375	9.5	0.297	7.50	26.73	34.23

Note: 1. Caustic soda costs P 10.00/liter; 1 liter caustic soda contains approx. 50% NaOH by weigh Cost/kg NaOH = P 20.00/kg

2. Calcium hypochlorite costs P 90.00/kg

\* Grams Dietary fiber / .100 grams dry bagasse pith

\*\* Dietary Fiber

Table 5. Cost estimate of chemicals used in the isolation of dietary fiber from bagasse pith.

Run No.	% Yield *	Kg Bagasse per kg DF**	Total % NaOH g NaOH	Kg NaOH per kg DF	Total % Ca(OCl) <sub>2</sub> g Ca(OCl) <sub>2</sub>	Kg Ca(OCl) <sub>2</sub> per kg DF	Cost of NaOH (P)	Cost of Ca(OCl) <sub>2</sub> (P)	Total Cost/kg DF (P)
			----- 100 g bagasse		----- 100 g bagasse				
1	38	2.632	24.00	0.632	12.00	0.316	12.64	28.44	41.08
2	30	3.333	11.25	0.375	11.25	0.375	7.50	33.75	41.25
3	29	3.448	12.00	0.414	8.00	0.276	8.28	24.84	33.12
4	30	3.333	9.00	0.300	8.00	0.267	2.48	24.03	26.51
5	32	3.125	8.00	0.250	8.00	0.250	5.00	22.50	27.50

Note: 1. Caustic soda costs P 10.00/liter; 1 liter caustic soda contains approx. 50% NaOH by weigh Cost/kg NaOH = P 20.00/kg

2. Calcium hypochlorite costs P 90.00/kg

\* Based on g dietary fiber per 100 grams dry bagasse pith

\*\* Dietary Fiber

Table 6. Proximate analysis of dietary fiber (Dry basis).

Raw Material	% H <sub>2</sub> O	% Lignin	% Alpha-cellulose	% Hemi-cellulose	Density	Water-Holding Capacity*		KMnO** No.	pH** (2 % soln)
						By Suction	By-Centri-fugation		
Whole Bagasse	6.0	0.60	56	43	0.10	3.5	8.0	2.5	8.4
Bagasse Pith	8.0	0.40	55	44	0.16	4.0	10.0	3.0	8.2

\* Two analytical methods were used: AOAC method for Peat & Method by Mc Connel et al (1974), respectively

\*\* Measure for bleachility/whiteness

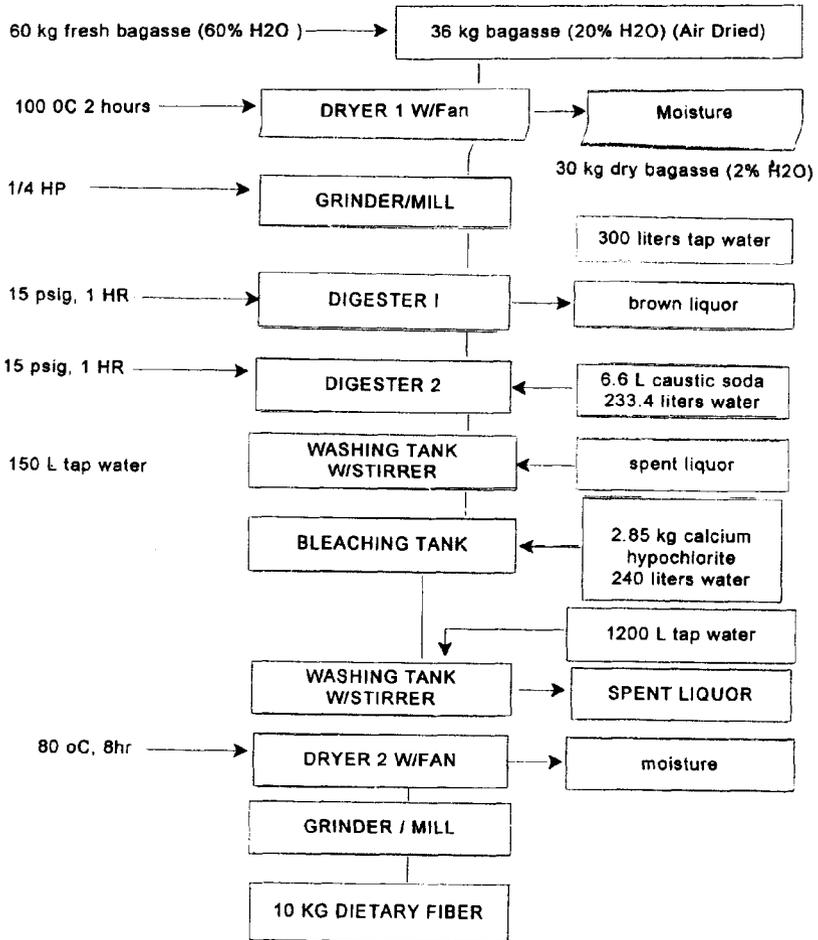
\*\*\* pH of deionized water used is 8.4

Table 7. Percentage composition of chemical residues in dietary fiber. (Analyzed by the Bureau of Food and Drug).

Component/Analyze	% Component in Dietary Fiber
Sodium	0.0076
Chloride ( as NaCl)	0.86
Calcium	2.81
pH	8.21

**Table 8. Process flow sheet for the production of dietary fiber from whole bagasse.**

**Basis: 10 kilogram dietary fiber**



# PASSIVE SOLAR ENERGY SYSTEM FOR CROP AND FOOD DRYING

*Samuel S. Franco, Reynaldo C. Castro, Carlo S. Sambo,  
Heraldo L. Layaoen, Percival O. Libed, Jr.,  
Jerry C. Ragudo and Benjamin S. Mercado, Jr.  
Mariano Marcos State University  
Batac, Ilocos Norte, Philippines*

## ABSTRACT

The use of a modified passive solar energy system was evaluated for food and crop drying. A natural convection system was constructed as a drying kiln with walls used as solar collectors. Polyethylene plastic was used as a glazing material. The solar heating system used involved radiation and convection heat transfer system. An auxiliary heating system was provided using fuelwood and other biomass fuels. Provision of sliding trays makes the barn suitable for drying other crops using 100% of solar energy. Applications include fruit and glaze, fish and sea weeds drying.

Result of the testing and evaluation indicates that depending on the kind and type of products solar energy contributes as much as 70% to 100% of the total energy required in drying. The use of auxiliary heating system only comes in when the required temperature is 50°C or above. The temperature inside the structure gets even higher than the required drying temperature, thereby needing a mechanism to cover the collector.

The system is simple and does not require fans nor control system. It can be easily installed in a farm because it is made of locally available materials. The use of the system requires no change in the existing post-production system practiced by local farmers.

## INTRODUCTION

The development of Multi-Crop Solar Dryer (MCSD) in Ilocos is ideal for three reasons. First, because the limited arable land and dense population, the cropping system in the region is very intensive. Second, the crops grown especially tobacco, require huge amounts of energy to cure or dry. In fact, because tobacco curing uses a lot of fuelwood, the denudation of forests in the area and in the neighboring regions has been blamed on the tobacco industry. Lastly, the region receives the highest solar insolation in the country.

Solar dryers are not new technologies. During the peak of the energy crisis in the 70s, many models were developed. Most of these models, however, are of the active type which require blowers to circulate the drying air, electricity to run these blowers, and relatively sophisticated controls. An example of this type of solar dryer is the Taiwanese-designed solar curing barn put up during the late 70s in Ilocos Norte by the then Philippine Virginia Tobacco Administration. Because

of the high initial and maintenance costs and completely different method of operation from what the farmers were currently following, it was never adapted. This experience proved that what the farmers need is a simple, low-cost dryer with a method of operation within their level of technical competence. It is towards this need that the development of the NTA MCSD was committed.

## METHODOLOGY

### Development of the Multi-Crop Solar Dryer (MCSD)

The MCSD development began in the early 80s (Castro et al., 1989) when a solar-aided curing barn prototype for fuel-curing tobacco was built. It was low in cost, simple in design, made of locally available materials and the method of operation needed was familiar to the farmers. The prototype had a dimension of 3.0 x 5.34 m on the base and 1.83 x 5.34 m on top. The overall height was 3.05 m. It had an effective volume of 29.8 m<sup>3</sup>, enough to accommodate the tobacco produced from 0.33 to 0.5 ha. The roof and walls, which were made of corrugated galvanized iron (G.I.) sheets were painted flat black and provided with clear polyethylene plastic glazing which served as solar collectors. The structure was oriented with its longer walls facing the east-west direction.

Since the amount of energy collected and retained was determined by the collector glazing, several configurations were tested. From 1980 to 1981, single layer polyethylene sheet (1 mm thick) was used. Two layers were tested during the 1982-1983 and a panelized double-layer cover was used from 1983-1984 to 1985-1986 curing seasons. The panelized version facilitated setting-up of the glaze during drying and its removal for storage after the drying operations.

During the 1985-1986 season holding trays for grains and legumes were added to the structure and trials were conducted to use the solar barn for drying other crops. Results from limited tests showed the barn to be effective in drying other crops.

In 1988, the MCSD was packaged for commercialization through the support of the ASEAN-Canada Project on Solar Energy in Drying Processes. Actual implementation of the project started in 1992.

### Design Specification and Method of Operation

The final MCSD has a dimension of 3.72 x 6.00 m at the base and 2.00 x 6.00 m at the top. The overall height is 3.90 m. It has an effective volume of 46.8 m<sup>3</sup>, enough to accommodate the tobacco produced from 1.0 ha.

To meet the condition of simplicity in design and operation, a passive greenhouse type collector system was adapted as in the earlier models. The roof and the east and west walls were utilized as collectors. Unlike in the earlier models, the north and south walls were not used as collectors but were insulated with non-cross linked polyethylene foam. It was also more airtight to minimize heat losses due to infiltration. Gravel beds were also placed on the floor to serve as heat storage medium.

Figure 1 summarizes the operation of the MCSD. Solar energy strikes the black walls and roof through the plastic glazing and is absorbed. This energy is transferred inside the dryer mainly through radiation and convection. Some of this energy is stored as reserve energy in the gravel beds as well as on the roof and walls. When collected and stored solar heat cannot meet the requirements of curing/drying fuelwood provides supplemental energy through the auxiliary heating system.

### **Thermal Behavior and Drying Tests**

Two sets of test were done each year to evaluate the performance of the MCSD. The first set determined the heat collection and storage efficiency of the MCSD while the second set was for crop curing and drying.

#### **Heat Collection and Storage Tests.**

Data were collected with no load and with the ventilators closed. Solar radiation, ambient temperature and the temperature inside the MCSD were monitored every hour for a 24-hour period. During the 1993-1994 curing season the data gathering system was computerized. The data acquisition system used was equipped with a pyranometer, thermocouple wires, and relative humidity (RH) meters. Hence, solar insolation, temperature and humidities at different points inside and outside the MCSD, were accurately measured.

#### **Curing and Drying Tests:**

Drying of chopped garlic, corn (on cob) and soybeans, were performed during the 1993-1994 season. The products were placed on trays specially made for the MCSD. The products were dried using solar energy.

For tobacco curing, leaves of uniform maturity were used as samples. The general curing procedure was followed, that is, maintaining a gradual increase in temperature from 28 to 45°C at 80 to 90% RH during the yellowing phase from 46 to 60°C at 30 to 80% RH during the color fixing, and from 61 to 77°C at 20 to 30% RH during midrib drying. Fuelwood consumption was closely monitored. From 1980 to 1984, the curing performance of the MCSD was compared with a concrete conventional barn. For the 1993-1994 season, galvanized iron barns

(insulated and uninsulated) were used.

## RESULTS AND DISCUSSION

The manual recording system used to monitor solar insolation and wind speed during the early years limited the data collection, however, a distinct trend indicated a direct relationship between the ambient and the MCSD's internal temperature. Figure 3 shows the relationship of the ambient and MCSD temperatures over the 1980-1981 to 1983-1984 curing seasons. The figure indicates the relative effectiveness of the type of solar collector glazing in collecting heat. The 1980-1981 curve represents the collector with single layer continuous (SLC) plastic cover. The other two curves represent double layer continuous (DLC) and double layer-panel (DLP) collector covers for 1982-1983 and 1983-1984 curing season, respectively.

The interior temperature was always higher than the ambient during daytime. The solar collector with SLC was more effective in collecting solar heat. With an average MCSD temperature increase of 3.1°C per unit increase in ambient temperature. The DLC cover type showed the lowest gain with an average increase only 2.6°C per unit increase in ambient temperature.

The higher rate of temperature increases inside the MCSD with SLC was due to the greater amount of solar energy passing through the single layer plastic glaze. The plastic has transmissivity of 0.9, and doubling the plastic layer decreased the transmitted energy by 19 percent.

During the 1982-1983 trials, the heated plastic cover softened causing it to sag to the collector plate. This was a greater problem with the DLC. This made the solar collector ineffective. This problem was solved by paneling the double plastic sheets.

Translating the temperature inside the MCSD to heat, the SLC collects an average of 13.61 MJ/day. The double layer cover generated 5.47 MJ/day for DLC and DLP, respectively (Table 1).

### Heat Conservation Efficiency

An indirect method was used to evaluate the heat conservation efficiency of the MCSD. The ability of the structure to maintain higher temperature at night without energy source indicates a high heat conserving efficiency. This maybe attributed to insulating property of the plastic sheet. The ambient and barn temperature gradient at night was used as the indicator of the heat conservation efficiency. As in the energy collection analysis, prediction equations based on the data until the 1983-1984 curing season were developed and the prediction curves are shown in Figure 2. DLP exhibited higher temperature during night time. This indicates higher conservation efficiency over the other cover types.

The SLC cover losses the greatest energy as evidenced by an average temperature drop of 2.3°C per unit decrease in ambient temperature. The double layer

covers decreased 1.1C for the DLC and 1.4C for the DLP. The significantly better heat conserving capacity of the double-layer covers maybe due to the insulating effect of the trapped air between the plastic layers. Furthermore, the DLC retained its original position during night time thereby giving comparable insulating effect as the DLP.

The detailed energy analysis currently undertaken using the 1993-1994 data provides deeper insights on the thermal behavior of the MCSD.

### **Drying of Soybean:**

The soybeans were placed on the trays to dry. The initial moisture content of 9% was reduced to 7.8% in six hours (Table 2). The ambient condition during the drying process was 33.51°C and the temperature of the drying chamber of the MCSD was 45.61°C, a difference of 12.09°C. The solar radiation during the drying period was 873.68 W/m<sup>2</sup>. The drying curve of the product is indicated in Figure 4. While the drying curve does not strictly follow the falling curve situation, the removal of moisture averaged 0.2% reduction per hour of drying.

The quality of the soybeans dried in the MCSD was better than sun dried samples. Cracks were avoided and it was more sanitary.

### **Drying of Chopped Garlic:**

Chopped garlic for powder normally takes about 30 days to dry to 15% moisture under the sun. This was reduced to 76 hours (Table 3). This decrease in moisture content was attained even at a lower solar radiation of 552.70 W/m<sup>2</sup> compared to the conditions when soybean was dried.

The drying curve of chopped garlic in the MCSD is shown in Figure 4. During the 76 hours drying period the average rate of moisture removal was 0.60% per hour. Comparing it to sun drying, the rate of moisture content reduction is 0.60%/ hour.

### **Drying of Corn Ears:**

Corn ears that were harvested during the wet months were dried in the MCSD. The result of the testing is indicated in Table 4. During the drying process, the difference in temperature between the MCSD to that of the ambient was 14.8C and solar radiation was 638.10 W/m<sup>2</sup> on the average. The moisture content was reduced from 29% to 19.67% in 33 hours (Figure 6). The average moisture content reduction was 0.28% per hour. The moisture content of 19.67% is suitable for shelling the corn.

The use of the MCSD has the advantage over the sun drying during that period because of occasional rain.

### **Tobacco Flue-Curing:**

The MCSD can also be used in the flue-curing of tobacco. While the amount of solar energy is enough for the initial stages of flue-curing, supplemental energy from fuelwood is needed at the later stages. The high temperature of 70°C required at the later stages cannot be fully supplied by solar energy. In terms of energy savings, the MCSD can reduce the used of fuelwood by as much as 50% compared to that of the traditional concrete barn of the same capacity (Table 5). Comparing it with a galvanized iron barns with and without, a fuelwood savings of 29.64 and 54.75% respectively were realized (Table 5). Based on the survey of Recta (1983), the MCSD can reduce fuelwood consumption by 83.91 and 88.86 for uninsulated clay barn with single pass flue system and uninsulated concrete barn with single pass flue-system, respectively.

The quality of the cured leaves dried in the MCSD is comparable to that of the concrete barn. The MCSD does not require a change in the system of post-harvest operations for tobacco.

### **Financial and Other Advantages**

The cost of materials for constructing an MCSD good for a hectare of tobacco is P66,052.00 whereas a concrete barn of similar capacity would cost P76,644.00. Hence, the MCSD is even cheaper by P10,592.00 compared to a conventional barn. The 50% savings in fuelwood would also mean an additional income of P2,000.00 for the farmers. The savings in fuelwood-use also means a big positive contribution to environmental conservation.

Furthermore, the capability of the MCSD to dry other crops with free energy from the sun advantage over the conventional barns using fire wood. In the drying of the food crops, the use is liberated from the tedious work of gathering the product during rains and at night. The process is also more sanitary because the drying chamber is enclosed.

### **SUMMARY AND CONCLUSION**

Compared with other types of solar dryers, the multi-crop solar dryer has the advantage of lower cost and simple design. It does not require a drastic change in procedure from the traditionally used farmer's barns. It is a passive system utilizing its black walls and roof as solar heat collector. Thermal storage is provided by the rock bed on the barn floor.

The MCSD can reduce fuelwood consumption by as much as 50% in flue-curing tobacco. The quality of cured leaves was similar to those cured using the traditional barns. For the drying of the crops like soybeans, corn and garlic, solar energy alone is sufficient to dry the product.

Financial analysis indicated that the MCS D has cost advantage over that of the concrete barn of the same capacity. It is also more sanitary as the crops are not exposed to the elements.

#### REFERENCES:

- Castro, R. C., Franco, S. S., Glova, A. D., Recta, F. R., Dilla, E. M., Austria, A. T. and Dulay, J. T. 1989. Utilization of Non-Conventional Energy Sources in Tobacco Curing and Drying of Other Crops. Second ASEAN Science and Technology Congress Proceedings. 2:590-605.
- Recta, F. R. 1987. Characteristics of Flue-curing barns in Ilocos Norte. MS Thesis. University of the Philippines at Los Banos. Unpublished

**Table 1. Estimated usable energy collected by the MCSD.**

Year	Type of Glazing	Estimated Usable Energy Collected, (MJ/day)
1980 - 1981	Single layer, continuous	13.61
1981 - 1982	Double layer, continuous	5.47
1982 - 1983	Double layer, panelized	12.39

**Table 2. Drying experiment for soybeans in the MCSD.**

Time (minutes)	Moisture Content (% wet basis)	Temperature, C		Solar Radiation (W/sq.m.)
		Ambient	MCSD	
0.00	9.00	33.40	43.50	9950.20
20.00	8.91	33.40	43.50	950.20
50.00	8.82	33.90	44.50	972.90
80.00	8.75	33.90	45.00	955.60
110.00	8.73	34.30	44.20	999.50
140.00	8.54	34.10	44.50	1027.80
170.00	8.27	34.30	45.20	960.60
200.00	8.17	33.60	46.90	1035.10
260.00	8.08	32.80	48.10	778.80
320.00	8.08	32.60	47.80	571.60
380.00	7.80	32.30	48.50	408.20
Mean		33.51	45.72	1691.86

**Table 3. Drying experiment for garlic (chopped) in the MCSD.**

Time (hour)	Moisture Content (% wet basis)	Temperature, C		Solar Radiation (W/sq.m.)
		Ambient	MCSD	
0.00	60.00	25.82	33.59	188.05
3.00	57.00	32.57	38.20	746.84
8.00	44.00	31.70	50.80	560.00
24.00	31.36	31.99	46.00	598.80
52.00	25.31			
72.00	16.46	32.38	42.49	707.20
76.00	14.97	31.41	39.49	415.32
Mean		30.96	41.76	536.04

**Table 4. Drying experiment for corn (in cobs) in the MCSD.**

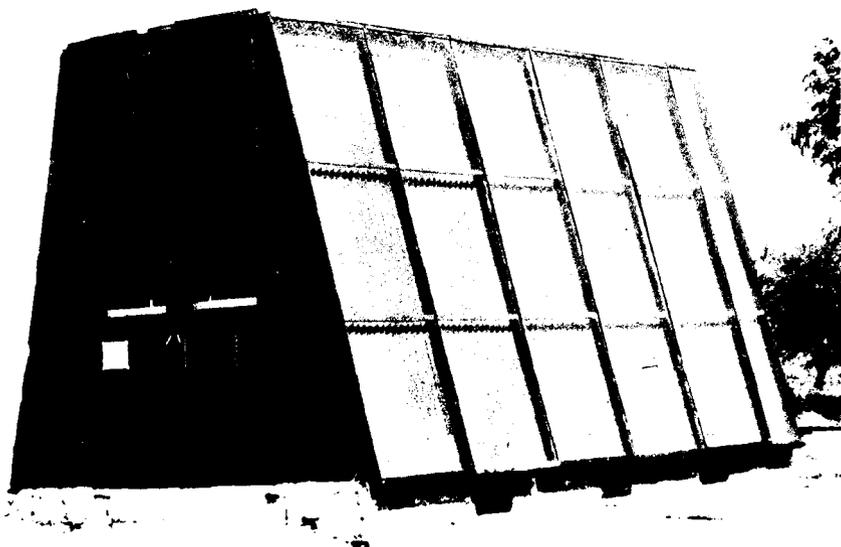
Time (hour)	Moisture Content (% wet basis)	Temperature, C		Solar Radiation (W/sq.m.)
		Ambient	MCSD	
0.00	29.00	30.90	44.40	536.00
2.00	28.87	31.05	36.95	613.98
4.00	28.09	31.74	50.64	953.59
6.00	27.22	31.81	51.99	973.34
8.00	25.58	28.43	51.55	583.88
24.00	23.18	26.05	31.58	56.03
26.00	22.18	31.31	53.41	773.90
28.00	21.32	30.90	44.85	685.96
33.00	19.67	28.11	37.86	566.28
Mean		30.03	44.80	638.11

**Table 5. Fuelwood savings of the MCSD against conventional curing barns in flue-curing tobacco.**

Cure Number	Fuelwood savings compared to conventional curing barns, %.			
	1	2	3*	4*
1	51.83	25.10	82.87	88.14
2	52.13	25.57	82.98	88.21
3	52.13	25.57	82.98	88.21
4	56.32	32.08	84.47	89.24
5	56.32	32.08	84.47	89.24
6	57.25	33.53	84.80	89.47
7	57.25	33.53	84.80	89.47
Mean	54.75	29.64	83.91	88.85

Notations:

- 1 Galvanized Iron Walled Barn without insulator.
  - 2 Galvanized Iron Walled Barn with Insulator.
  - 3 Uninsulated clay barn with single pass flue-system.
  - 4 Uninsulated concrete barn with single pass flue system.
- \* Based on the survey of F. R. Recta, 1983.



**Figure 1. The Multi-crop Solar Dryer.**

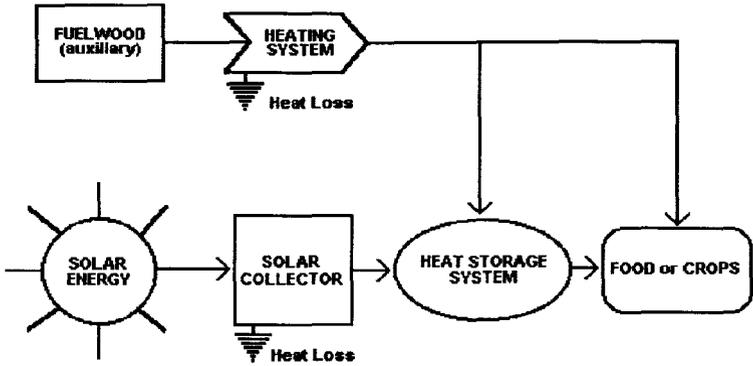
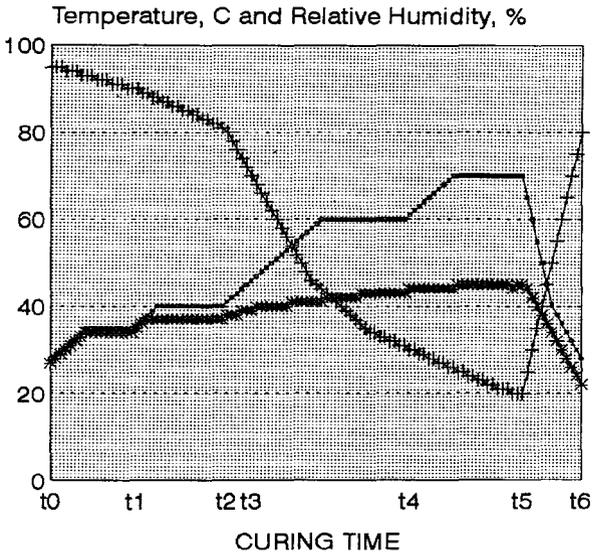


Figure 1. Energy flows in the Multi-Crops Solar Dryer.

# FLUE-CURING CHART



PARAMETERS

- Dry-Bulb Temperature, C
- + Relative Humidity, %
- \* Wet-Bulb Temperature, C

Figure 2. Thermal behavior of the Multi-Crop Solar Dryer.

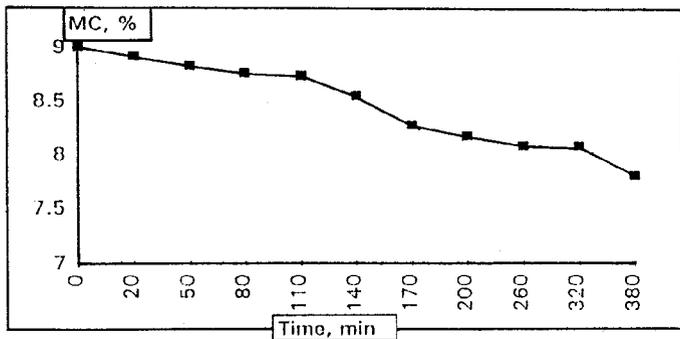


Figure 3. Drying curve of soybean in the MCSD.

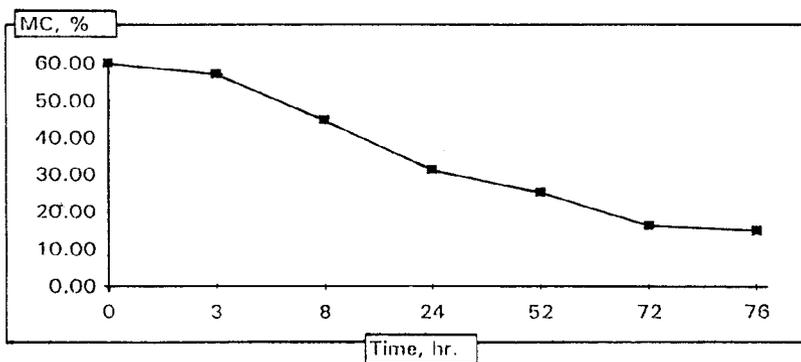


Figure 4. Drying curve of garlic (chopped) in the MCSD.

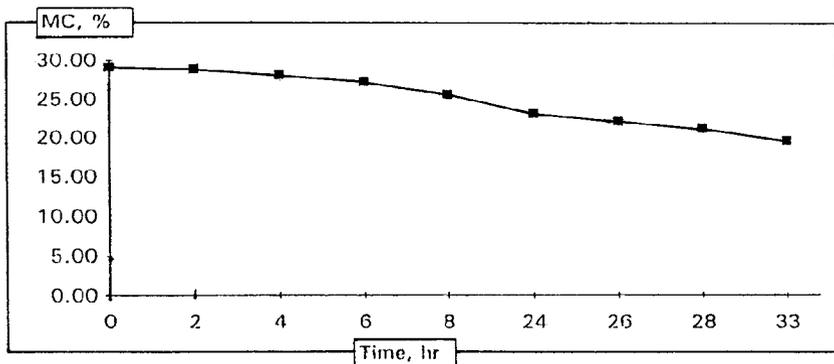


Figure 5. Drying curve of corn in the MCSD.



# **FRUIT AND VEGETABLE PROCESSING TECHNOLOGIES FOR VILLAGE-TYPE PROCESSING PLANTS**

*Nerius I. Roperos, Virginia TD. Pacaba and Lydia M. Zara  
Bureau of Plant Industry, Department of Agriculture*

## **ABSTRACT**

In the Philippines, the fruit and vegetable processing industry is characterized by the dominance of cottage and small scale establishments. Their products have varying outlets, many are sold in groceries and supermarkets while some are exported.

Processed fruits and vegetables in various forms remain to be among the country's regular and promising exports. Production and processing of most of these products could be done using simple equipment in rural households. The Bureau of Plant Industry has developed technologies for some products which have possible application for village level processing.

## **INTRODUCTION**

Fruit and vegetable processing eliminates post-harvest wastage and spoilage, provides better returns to growers, and assures consumers of supply all-year round, thus playing a major role in the attainment of food self-sufficiency.

This paper will attempt to present an updated situationer on the fruit and vegetable processing industry in the rural areas in the Philippines, data on exports of processed fruit and vegetable products, technologies developed at the Bureau of Plant Industry and their possible application to village-type processing plants, and advantages of putting up village-type processing plants.

## **THE FRUIT AND VEGETABLE PROCESSING INDUSTRY IN THE RURAL AREAS**

In the Philippines, the fruit and vegetable processing industry is characterized by the dominance of cottage and small scale traditional establishments located mostly in the rural areas. In these establishments processing operations differ in their level of technology. They are often family-owned, staffed mostly with women workers.

By 1988, the registered food manufacturing establishments totalled 33,429 as shown in Table 1. Classified based on employment size, 93.73% or 31,335 belong to the cottage level with less than 10 employees. The rest of the industry constitutes the following: 1,864 or 5.58% for the small-scale, with 10-99 workers; 83 or 0.25% for the medium, with 100-199 workers; and 147 or 0.44% for the large, with 200 or more number of workers. The industry is described as

those establishments engaged in canning and preserving fruits and vegetables; fruit and vegetable juices; fruit and vegetable sauces; peanut and peanut products; cassava/arrowroot flour, starch and its products.

A wide range of low technology processing equipment are used since little capital is invested in machinery. Some entrepreneurs improvise with rudimentary equipment and some use secondhand or locally-fabricated equipment. Among the simple processing equipment commonly found are gas-fired cooking kettles, roasters, mechanical grinders, blowers, cutters, drying trays, ovens, and aluminum tables. There are manufacturer/exporting firms who do not invest in their own machinery but prefer to lease facilities. However, other successful manufacturer/exporting firms invest in machinery, like the Lubag Food Corporation located in Morong, Rizal, which manufactures assorted heat-processed products like nata de coco, coco juice, kaong, fruit cocktail, pickled vegetables, tomato sauce, sweet corn, purple yam, jam and novelty products like heart of palm, banana blossoms and green jackfruit. Currently installed in its plant are equipment like can seamer, retort, slicer (mobile), steam jacketed kettles and exhaust box. Scheduled for installation are equipment obtained through a government financing loan. These are dehydrator, filling machine, pulper, crusher, and boiler.

In Lobo, Batangas, town located 116 km. away from Manila, candied/glazed tamarind is being processed in a crude way. Cooking is done in large cast-iron vats using firewood as fuel. The cooked fruits are drained, arranged in drying trays and sun-dried atop the building with galvanized roofing. The dried fruits are packed in wax-lined boxes and covered with cellophane. A wholesaler takes charge of the marketing and distribution in groceries and supermarkets almost nationwide.

Batch type processing and packaging is employed with minimum equipment. Polyethylene is the most common type of packaging material. For heat-processed products, bottling is widely practiced, using recycled bottles with new caps.

### Marketing

These products have varying outlets. Many are sold in groceries and supermarkets. Some primary processed foods are sold to large food processors as ingredients for their operations, e.g. fruit purees to ice cream and to fruit juice manufacturers. There are also cottage and small scale processors who export processed fruit and vegetable products.

## EXPORTS OF PROCESSED FRUIT AND VEGETABLE PRODUCTS

Processed fruits and vegetables in various forms remain to be among the country's regular and promising exports. Table II shows the quantity and value of the different processed fruit and vegetable exports for a 3 year period, from 1991-1993. There was a 32.19% increase in the quantity of exports from 1991

(259,707.6 kg) to 1992 (383,048.9 kg.) and a 21.04% decline from 1992 (383,048.9 kg.) to 1993 (316,469.1 kg.). Also shown in descending order were the commodities exported in 1993, as follows: fruits in syrup which tops the list; dried rootcrops; juices, concentrates, purees, and pastes; other food preparations which include banana chips/crackers, banana ketchup and flour, flour meal and powder of fruit and nuts; food preserves like beans, nata de coco, macapuno, kaong; fruits and vegetables in vinegar/acetic acid; dried fruits; jams, jellies and marmalades; nuts (peanut); dried vegetables and legumes; and fruits in brine/frozen.

The United States is the major foreign market for pineapple syrup, concentrates and juices. There is also a growing trend in the exportation of dried fruits. In the United Kingdom, Germany and France, bananas and papayas are the most popular among the tropical dried fruits, used as snack food and as ingredients in breakfast cereals, ready-to-eat meals and desserts.

### **TECHNOLOGIES AVAILABLE ON PROCESSED FRUITS AND VEGETABLES**

Despite its simple facilities and equipment, the Laboratory Services Division of the Bureau of Plant Industry continuously develops processing technologies for indigenous fruits and vegetables.

Listed in Table III are selected crops with their corresponding scientific name and developed technologies which have possible application for village level processing. As indicated, simple and less costly processing techniques has been developed on the following food products: candy, jelly, jam, pickle, nectar, powder, sauce, chips, crackers, wine, catsup or simply dried and canned in syrup.

Production and processing of these products could be done using a simple equipment in rural households.

### **ADVANTAGES OF ESTABLISHING VILLAGE-TYPE PROCESSING PLANTS**

Different sectors in the rural areas abound with seasonal crops which generally find its way to local markets for consumption in the fresh state. At the peak of production, surplus go to waste with inevitable losses. Establishment of village level processing plants which could be integrated with the production activities of the area would minimize post-harvest losses. Several positive factors could also be generated like employment opportunities for the rural population, thus preventing migration from rural to urban areas. This would also result to increased farm income, improved diet, help overcome seasonal gluts and shortages of produce, thus contributing to overall integrated rural development.

To further stress the point, let me quote from a FAO Paper on Enhancing Rural Employment and Incomes Through the Development of Agro-Processing

Industries. "Dispersal of rural cottage industries and simple processing plants throughout the countryside, close to the supply of raw material, drawing labor from the farming community, increases linkage between agriculture and industry".

Needless to say, food processing plants could only be established in areas with adequate potable water and electricity. Energization is continuously being done. As of May 1994, the National Electrification Administration reported that out of 41,910 barangays (COMELEC - May 1994 elections) 23,153 barangays had been energized. On the other hand, the Local Water Utilities Administration (LWUA) reported that 583 water districts had been formed nationwide as of December 1993.

**Table 1. Number of food manufacturing establishments\* by employment size, 1988 (Food group).\*\***

Employment size	No. of worker	No. of manufacturing establishment	%
Cottage	Less than 10	31,335	93.73
Small	10 – 99	1,864	5.58
Medium	100 – 199	83	0.25
Large	200 or more	147	0.44

\*Industry Description – Food Manufacturing (canning and preserving fruits and vegetables; fruit and vegetable juices; fruit and vegetable sauces; peanut and peanut products; cassava flours; starch and its products).

\*\* Data Source – SME Statistical Report, Bureau of Small and Medium Business Development, Department of Trade and Industry, June 1992.

**Table 2. Quantity and value of processed fruits and vegetables exports. (1991-1993).**

COMMODITY	1993		1992		1991	
	Quantity (kg)	Value (\$)	Quantity (kg)	Value (\$)	Quantity (kg)	Value (\$)
Fruits in syrup	131,928,632	65,155,985	212,142,310	105,642,621	208,041,490	103,868,617
Dried rootcrops	116,018,630	2,702,592	58,574,063	8,344,657	38,888,427	5,341,735
Juices, concentrates, purees pastes	59,842,302	25,723,485	93,570,877	77,743,385	97,833,944	52,008,461
Other food preparation (chips/crackers; banana ketchup and flour; flour meal and powder of fruit and nuts)	7,102,091	6,636,230	12,241,473	13,867,759	13,652,619	15,500,979
Food preserves (beans, nata de coco, macapuno, kaong)	655,529	1,410,959	1,679,381	2,977,647	1,200,770	2,290,784
Fruits and vegetables in vinegar/acetic acid/frozen not frozen/salted and fermented	417,551	759,325	1,201,841	1,572,060	988,284	1,311,987
Dried Fruits	273,422	998,491	1,126,028	5,050,039	1,107,802	4,803,229
Jams, jellies and marmalades	149,478	293,227	1,762,329	1,559,661	1,157,759	1,040,398
Nuts (peanut)	62,399	190,341	268,148	608,298	288,627	664,211
Dried vegetables & legumes	15,261	20,365	123,798	111,134	408,610	103,102
Fruits in brine and frozen	3,768	12,136	358,811	1,081,435	312,146	925,657
<b>Total</b>	<b>316,469,063</b>	<b>103,903,136</b>	<b>383,048,979</b>	<b>218,558,696</b>	<b>259,787,605</b>	<b>187,859,760</b>

SOURCE: Central Bank of the Philippines (Bangko Sentral ng Pilipinas)

Table 3. Developed technologies from selected fruits, vegetables and rootcrops.

Crop (English Name)	Scientific Name	Technologies (Processed Products)
<b>Fruits</b>		
1. Banana	<u>Musa sapientum</u> var. Cavendish	Candied, catsup, flour, wine
2. Banana	<u>Musa sapientum</u> Linn. var. <u>Compressa</u>	Chips/crackers, flour
3. Phil. Lemon (Kalabansi)	<u>Citrus microcarpa</u> Bunge	Juice, syrup, candied rind
4. Guava	<u>Psidium guajava</u> Linn	Jelly, nectar, wine
5. Soursop	<u>Annona muricata</u> Linn	Nectar, jam, frozen
6. Jackfruit	<u>Artocarpus hetero-</u> <u>phyluss</u> Lamk.	Candied, canned in syrup
7. Mango	<u>Mangifera indica</u> Linn.	Canned in syrup, jam, pickled, dried, puree, nectar, leather
8. Papaya	<u>Carica papaya</u> Linn.	Nectar, jelly, jam, canned in syrup, candied, dried, pickled
9. Passion fruit	<u>Passiflora foetida</u> Linn.	Jelly, jam, syrup
10. Pineapple	<u>Ananas comosus</u> (Linn.) Merr.	Canned in syrup, jam, candied, dried, wine frozen
11. Rambutan	<u>Nephelium lappaceum</u> Linn.	Canned in syrup
12. Santol	<u>Sandoricum koetjape</u> (Burm. F.) Merr.	Canned in syrup/preserve candied, wine
13. Cassava	<u>Manihot esculenta</u> Crantz	Flour, starch
14. Yam (purple)	<u>Dioscorea alata</u> Linn.	Powder
15. Ginger rhizome	<u>Zingiber officinale</u> Rosc.	Instant ginger tea, dried
16. Soybean	<u>Glycine soja</u> L.Sieb. & Zucc.	Soysauce, tokwa, tahu
17. Tomato	<u>Lycopersicon lyco-</u> <u>persicum</u> L. (Karst)	Catsup, sauce, pickled, wine

**REFERENCES**

1. Crisostomo, Lydia C. "Fruit and Vegetable Processing in the Rural Areas". Paper presented during the Agri-Aqua Fair held at Philcote on May 30, 1988.
2. Commission on Elections. Statistics Department.
3. "Enhancing Rural Employment and Income Through the Development of Agro-Processing Industries". FAO Paper. Twenty-First Regional Conference for Asia and the Pacific. New Delhi, India, 10-14, February, 1992.
4. Food Composition Tables. 1990. Food and Nutrition Research Institute, Department of Science and Technology. Manila, Philippines.
5. Local Water Utilities Administration. 1993 Annual Report.
6. Lustre, Alicia O. and Caridad F. Aspiras. "Status of the Food Processing Sector" 1981. Food Map of the Philippines and How to Establish a Food Processing Business. Productivity and Development Center. DAP.
7. National Electrification Administration. Planning Department.
8. Preservation of Fruits and Vegetables. 1983. Laboratory Services Division, Bureau of Plant Industry, Department of Agriculture, Manila, Philippines.
9. SME Statistical Report. June 1992. Bureau of Small and Medium Business Development, Department of Trade and Industry, Manila, Philippines.
10. The Latest Developments and Improvements of Fruit and Vegetable Products. 1989. Laboratory Services Division, Bureau of Plant Industry, Department of Agriculture, Manila, Philippines.

# DRYING CHARACTERISTICS OF SWEET POTATO SLICES

*Lemuel M. Diamante  
Visayas State College of Agriculture,  
Baybay, Leyte, Philippines*

## ABSTRACT

The effect of drying temperature, air relative humidity, air velocity and sample thickness on the drying rates of sweet potato slices were studied. Results showed that the drying of sweet potato slices occurs only in the falling rate period. Among the factors and ranges considered, air velocity gave the most significant effect on the drying rates of sweet potato slices. The sample thickness and drying temperature had also significant effects on the drying rates. The factor with little effect on the drying rates was air relative humidity.

## INTRODUCTION

Drying of sweet potato roots preserves for several months at ambient conditions without the risk of spoilage. It can then be rehydrated and used as fresh substitute or milled into flour and used for a variety of purposes.

To design efficient dryers for sweet potatoes quantitative data are needed for drying rates under a range of drying conditions. As it is impossible to predict drying rates from heat and mass transfer theories alone, the drying rates must be obtained experimentally. The determination of drying rates are usually generated by batch drying experiments which involve monitoring moisture content ( $M$ ) with time ( $t$ ) under controlled conditions. The slope of tangent to the drying curve ( $dM/dt$ ) represents the drying rate of the sample at a particular moisture content or time. Taking the slopes over the entire curve, plots of drying rate against moisture content or time are obtained<sup>4</sup>.

There are already a number of published data describing the drying rates of a sliced biological materials under constant drying conditions<sup>1,2,3,7,9,10,11</sup>. However none is available on the drying rates of sliced sweet potato roots.

Hence this study was aimed at investigating the effects of drying conditions and slice thickness on the drying rates of sweet potato slices and come up with a recommendation for optimum drying of the material.

## MATERIALS AND METHODS

### Sweet Potatoes

Sweet potato roots were purchased from the local market. The roots were stored in a cabinet under optimum conditions of 15°C and 89% Rh<sup>6</sup>.

## Drying Equipment

The laboratory dryer (Figure 1) consisted of a centrifugal fan (Section 1), a heating section (Section 2), a steam injection section (Section 3) and a drying chamber (Section 4). The centrifugal fan was powered by a variable speed motor, which was used to control the air velocity. The heating section used a steam heat exchanger and on-off 1-kW heaters with a proportional temperature controller for temperature control. The steam injection section was used to control air humidity via manual control of two gate valves. The drying chamber consisted of a drying tray (185 x 175 mm) made of stainless steel mesh suspended from a weighing system. The weighing system consisted of a digital balance (Mettler PE 1600), an integrator unit (Mettler GE 305 Lab Pac) to smooth out fluctuations in weight due to the air flow, a thermal printer (Mettler GA 44) and timer. This arrangement allowed a virtually continuous monitoring of sample mass with time. Dry bulb and wet bulb temperatures were monitored within the drying chamber.

## Drying Experiments

The laboratory dryer was set on the required drying conditions, and left for about an hour to reach steady state conditions (Table 1). Sweet potato samples were taken from the storage cabinet washed, peeled, trimmed and sliced to the required thickness (Table 1) using a mechanical slicer with a rotating blade. A sample of about 400 grams was evenly spread on the drying tray and covered with a wire mesh to prevent movement of slices as it dried. The tray was suspended in the drying chamber and the change in weight of the sample was monitored until the approximate moisture content was around 10% (dry basis). The average moisture content of the dried sample was determined by taking representative samples from the batch and oven drying until constant weight.

## Drying Rate Curve Determination

The weight of samples for the given time period was converted to moisture content by using the average weight of dry solids obtained from moisture content determination. The drying curve for each experiment was obtained by plotting the moisture content of the sample at the given time. Using the obtained drying curves of sweet potato slices the drying rate curves were determined as outlined in Diamante et al. (1990)<sup>4</sup>.

## RESULTS AND DISCUSSION

### Drying Rates of Sweet Potato Slices at Standard Conditions

Figure 2 shows the drying rate curves for five batches of sweet potato slices at the standard conditions of 60°C drying temperature, 12.5% air relative humid-

ity, 1.0 m/s air velocity and 6 mm sample thickness. The air relative humidity of 12.5% is the average value during the conduct of the experiment. The results showed that the drying of sweet potato slices occurs only in the falling rate period (FRP). There was no constant rate period observed in any of the drying runs. Similar observations were also reported by Islam and Flink (1982)<sup>10</sup> for potato, Vaccarezza et al. (1974)<sup>11</sup> for sugar beet and Chirife and Cachero (1970)<sup>3</sup> for tapioca.

The data were analyzed using the exponential model<sup>8</sup> following the procedure of Diamante et al (1990)<sup>4</sup> and the results are summarized in Table 2. The results suggest that the falling rate period of sweet potato slices consist of two stages with mean FRP drying coefficients of 0.183 and 0.258 hour<sup>-1</sup>, respectively. The mean critical moisture content of the samples was around 7% dry basis. The results suggest that the sweet potato slices dry slower in the first stage of FRP than in the second stage which also shown in Figure 2. The second stage in the FRP starts when the moisture content of the samples is around 7% dry basis.

#### Effects of Drying Temperature on Drying Rates

The drying rates of sweet potato slices obtained at different drying temperatures are presented in Figure 3. Results show that drying temperatures had a significant effect on the drying rates of sweet potato slices. The higher the drying temperature the higher the drying rates in both stages of the FRP. The results were similar to the observations obtained in drying sliced biological materials as reported by Fornell et al. (1980)<sup>7</sup> for several vegetables, Vaccarezza et al. (1974)<sup>11</sup> for sugar beet, and Chirife and Cachero (1970)<sup>3</sup> for tapioca.

During the falling rate period, the rate of diffusion is proportional to the sample surface temperature which is dependent on the drying temperature<sup>12</sup>. Therefore the higher the air drying temperature the higher the moisture diffusion out of the product.

#### Effect of Air Relative Humidity on the Drying Rates

Figure 4 shows the drying rates of sweet potato slices at different air relative humidity (RH). The results showed that air RH has little effect on the drying rates. The slight effect was observed at the first stage of the FRP for the low RH air. Bimbenet et al. (1985)<sup>1</sup> reported that lower humidity air gave higher drying rates at the first stage of the FRP for both carrots and potatoes. Diamante et al. (1992)<sup>5</sup> also noted that doubling the inlet air has little effect in the second stage of drying.

When the humidity of the air increases, the humidity driving force between air and the wet solid decreases thereby lowering the drying rates during the first stage of drying<sup>13</sup>.

### Effect of Air Velocity on the Drying Rates

The drying rates of sweet potato slices at different air velocities are presented in Figure 5. The data revealed that the air velocity had the most significant effect on the drying rates of sweet potato slices in both stages of the FRP among the factors and ranges studied. The lower the air velocities the lower the drying rates in both stages of the FRP. Similar observations were also reported by Bimbenet et al. (1985)<sup>1</sup> for carrot, Islam and Flink (1982)<sup>10</sup> for potato, Vaccarezza et al. (1974)<sup>11</sup> for sugar beet, and Chirife and Cachero (1970)<sup>3</sup> for tapioca.

From drying theory, it is expected that air velocity will only be significant in the CRP for a purely internal mass transfer resistance which occurs at air velocity above 2.5 m/s. However, in this study the lower air velocities were below 2.5 m/s and thus both external mass transfer resistance and air velocity will have a significant influence on drying behavior<sup>10</sup>.

### Effect of Sample Thickness on the Drying Rates

Figure 6 shows the drying rates of sweet potato slices at different sample thickness. The results suggest that sample thickness had a significant effect on the drying rates of sweet potato slices in both stages of the FRP. The thinner the samples the higher the drying rates in both stages of the FRP. Several investigators also reported similar findings such as Islam and Flink (1982)<sup>10</sup> for potato and Vaccarezza et al. (1974)<sup>11</sup> for sugar beet.

Under low air velocities as used in the study, both internal (sample thickness) and external (surface area per unit weight) mass transport resistance are significant<sup>10</sup>. Hence, the samples with thinner slices offered the least resistance to both factors resulting in higher drying rates in both stages of the FRP.

### Optimum Drying Conditions for Sweet Potato Slices

Based on the factors and ranges used in the experiments, suggest that in order to have faster drying of sweet potato slices it should have a sample thickness of 3 mm and drying conditions of 70°C drying temperature, 3.0 m/s air velocity and ambient relative humidity of around 12.5%. The drying rates of sweet potato slices may increase further by decreasing the sample thickness lower than 3 mm and using drying temperature higher than 70°C. However, increasing the drying temperature above 70°C may affect the physical and functional properties of the dried sweet potato and therefore need to be assessed first. There is no advantage in increasing the air velocity above 3.0 m/s since this is already above the boundary where a purely internal transport resistance controls and therefore no amount of airflow can increase the drying rate. Lastly it would be best to carry out the drying operations when there is lower ambient air relative humidity which usually occurs during hot and sunny days.

**REFERENCES:**

1. Bimbenet, J. J., J. D. Daudin and E. Wolfe. 1985. Air drying kinetics of biological particles. In *Drying '85* (R. Toei and A. S. Mujumdar, eds.). Hemisphere Publishing Corporation. New York. pp. 178-185.
2. Chiang, W. C. and J. N. Petrsen. 1985. Thin layer air drying of french fried potatoes. *Journal of Food Technology*. 20: 67-78.
3. Chirife, J. and R. A. Cachero. 1970. Through-circulation drying of tapioca root: *Journal of Food Science*. 35:364-368.
4. Diamante, L. M., P. A. Munro and M. G. Weeks. 1990. Moisture desorption isotherms and drying rates of mineral acid casein. In *Proceedings of the 18th AustralAsian Chemical Engineering Conference*. Auckland, New Zealand. August 27-30, 1990. Volume I. pp. 101-108.
5. Diamante, L. M., P. A. Munro and M. G. Weeks. 1992. Fluidized bed drying of a biological material (casein): Effect of drying conditions. Paper presented during the 42nd National Convention of PSAE held at Batac, Ilocos Norte on April 22-24, 1992
6. Edmond, J. B. and G. R. Ammerman. 1971. *Sweet Potatoes: Production, Processing, Marketing*. (AVI Publishing Company, Inc.). Westport, Connecticut.
7. Fornell, A., J. J. Bimbenet and Y. Almin. 1980. Experimental study and modelization for air drying of vegetable products. *Lebensmittel-Wissenschaft und-Technologie*. 14: 96-100.
8. Hustrulid, A. and A. M. Flikke. 1959. Theoretical drying curve for shelled corn. *Transactions of the American Society of Agricultural Engineers*. 3: 112-115.
9. Igbeka, J. C. 1982. Simulation of moisture movement during drying of a starchy food product - cassava. *Journal of Food Technology*. 17: 27-36.
10. Islam, M. N. and J. M. Flink. 1982. Dehydration of potato: I. Air and solar drying at low air velocities. *Journal of Food Technology*. 17: 373-385
11. Vaccarezza, L. M., J. L. Lombardi and J. Chirife. 1974. Kinetics of moisture movement during air drying of sugar beet root. *Journal of Food Technology*. 9: 317-327.

12. Watson, E. L. and J. C. Harper. 1988. Elements of Food Engineering. Van Nostrand Reinhold Company. New York.
13. Williams-Gardner, A. 1971. Industrial Drying. Leonard Hill. London

**Table 1. Drying conditions and sample thickness used in the experiments.**

Variable Studied	Temperature	Air Humidity	Air Velocity	Thickness
Standard Conditions	60°C	12.5% RH	1.0 m/s	6 mm
Effect of Temperature	50°C	12.5% RH	1.0 m/s	6 mm
	70°C	12.5% RH	1.0 m/s	6 mm
Effect of Air Humidity	60°C	10.0% RH	1.0 m/s	6 mm
	60°C	15.0% RH	1.0 m/s	6 mm
Effect of Air Velocity	60°C	12.5% RH	0.5 m/s	6 mm
	60°C	12.5% RH	3.0 m/s	6 mm
Effect of Sample Thickness	60°C	12.5% RH	1.0 m/s	3 mm
	60°C	12.5% RH	1.0 m/s	9 mm

**Table 2. FRP drying coefficients and critical moisture content for sweet potato slices dried at standard conditions of 60°C, 12.5% RH, 1.0 m/s and 6 mm thickness.**

Drying Run	First Stage (hr <sup>-1</sup> )	Second Stage (hr <sup>-1</sup> )	Critical MC (% db)
1	0.168	0.249	75
2	0.210	0.277	56
3	0.162	0.232	81
4	0.191	0.242	61
5	0.181	0.258	76
Mean with 95% confidence bounds	0.183±0.024	0.258±0.031	70±13

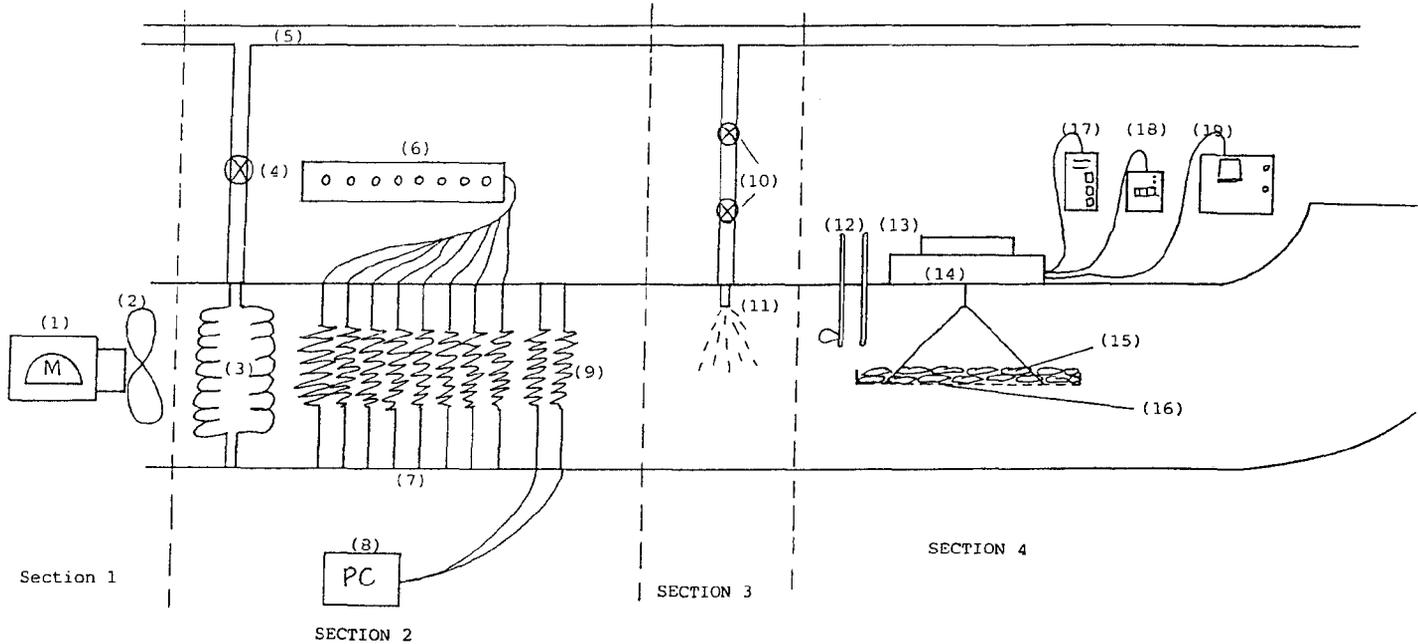


Figure 1. Schematic diagram of the laboratory tunnel dryer used in the experiments.

LEGEND:

- |                                  |                             |                          |
|----------------------------------|-----------------------------|--------------------------|
| (1) Variable speed motor         | (8) Proportional controller | (14) Weighing balance    |
| (2) Centrifugal fan              | (9) Two 1-kW heaters        | (15) Sweet potato slices |
| (3) Steam Heat Exchanger         | (10) Steam gate valves      | (16) Drying tray         |
| (4) Steam gate valve             | (11) Copper tubing          | (17) Integrator unit     |
| (5) Steam line                   | (12) Wet bulb thermometer   | (18) Timer               |
| (6) Bank of switches for heaters | (13) Dry bulb thermometer   | (19) Printer             |
| (7) Bank of 1-kW heaters         |                             |                          |

Figure 2. SP drying rate curves at standard conditions.

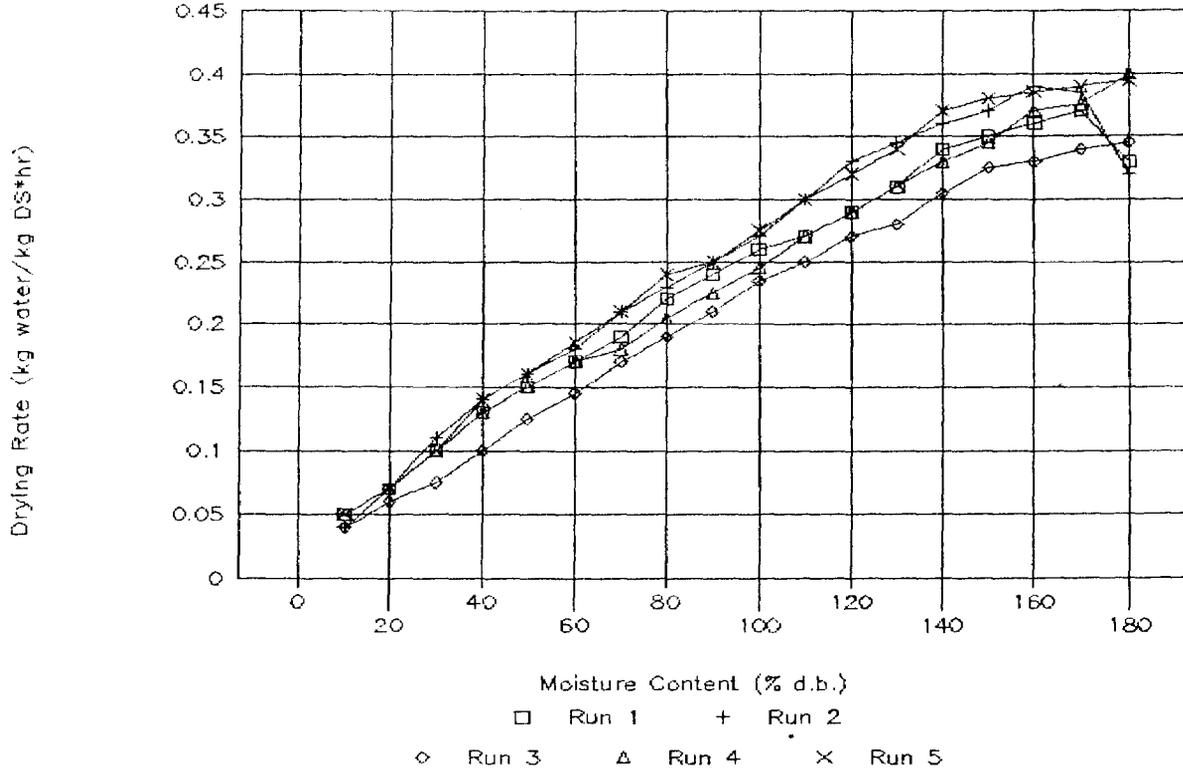


Figure 3. SP drying rate curves at different temperatures.

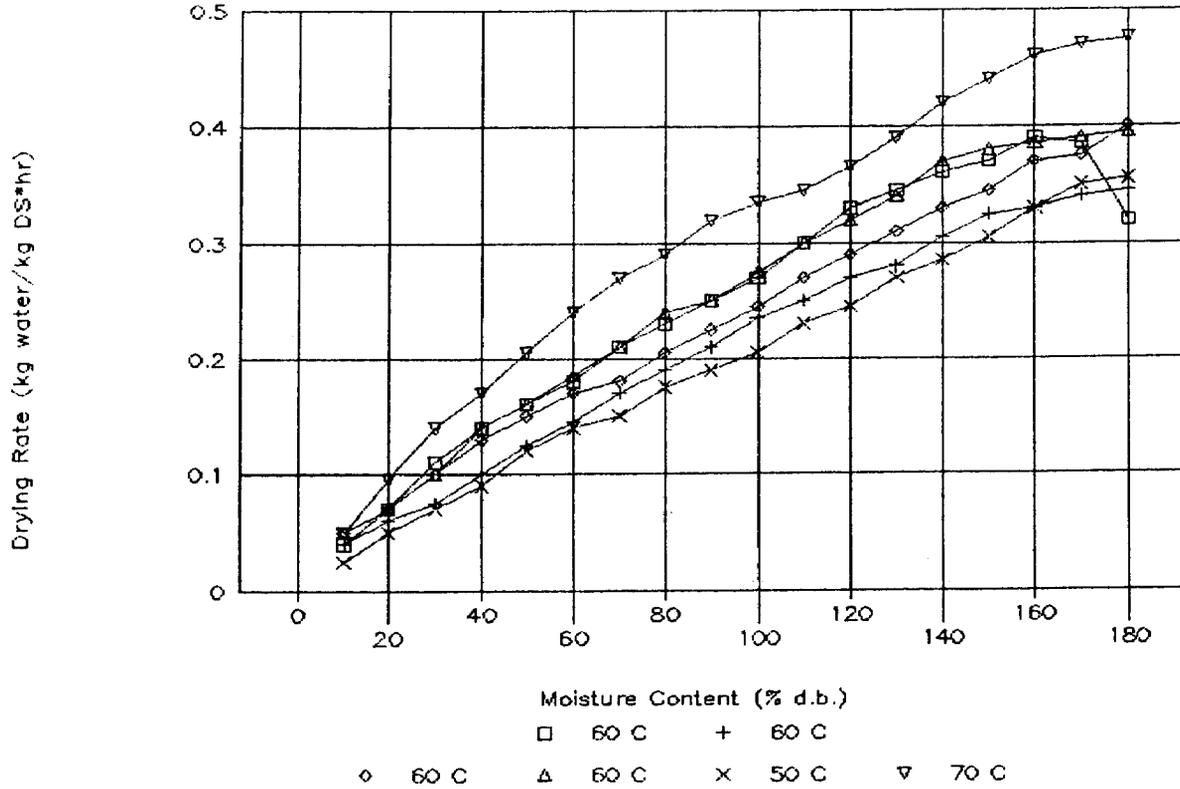


Figure 4. SP drying rate curves at different air humidity.

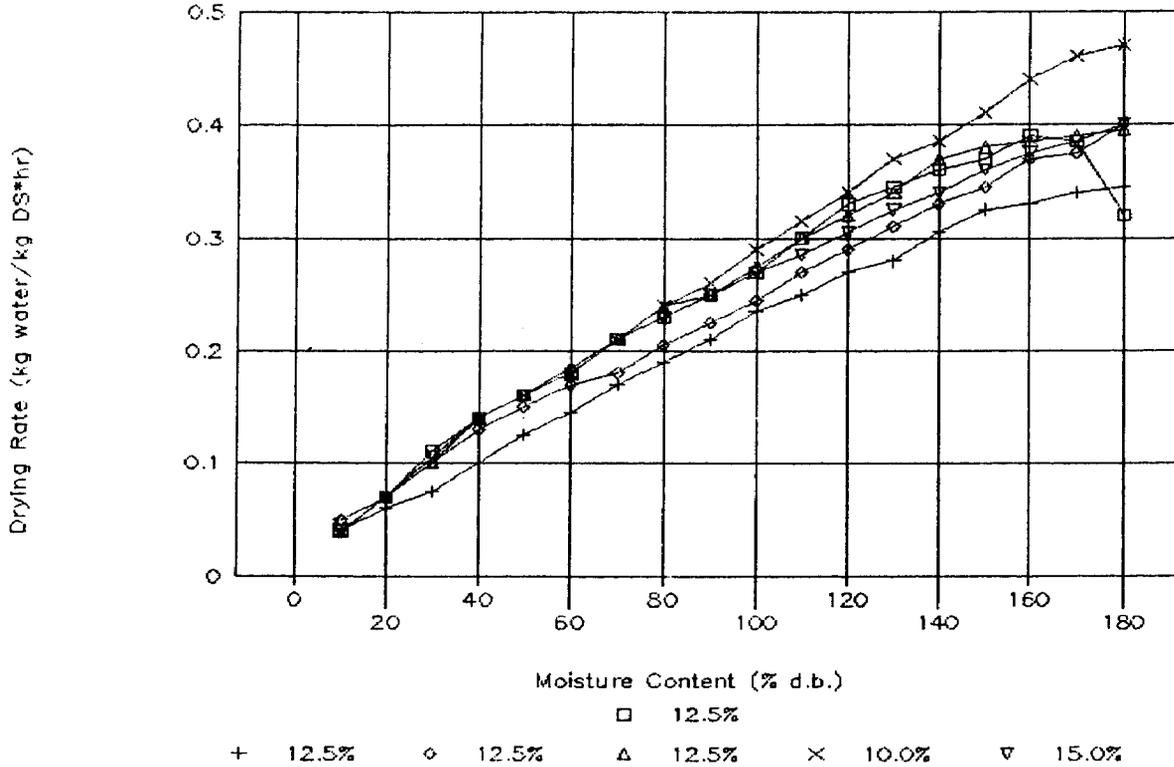


Figure 5. SP drying rate curves at different air velocity.

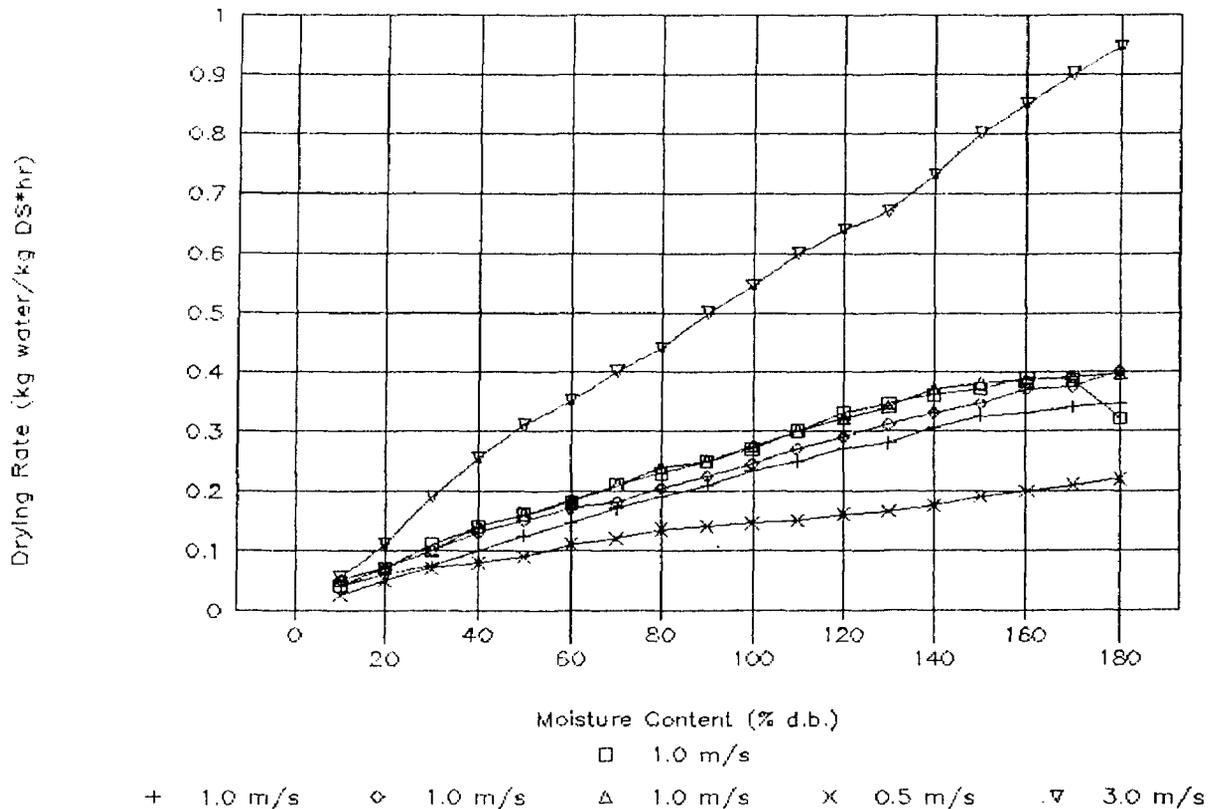
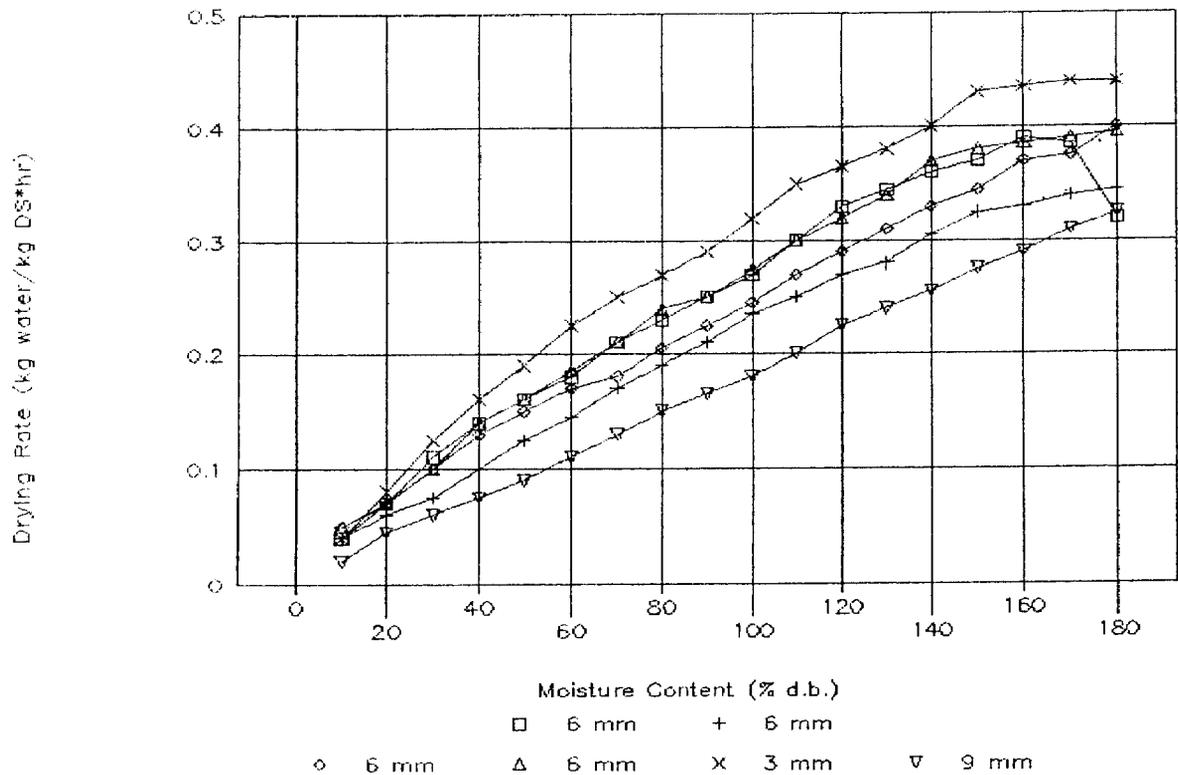


Figure 6. SP drying rate curves at different slice thickness.





# ESTABLISHMENT OF COMMUNAL FOOD PROCESSING CENTERS IN THE REGIONS

*L.M. Marero, A.M. Martin Jr., M.P.E. De Guzman;  
B.T. Molano, M.C. Cabagbag and W.N. Lainez  
Food and Nutrition Research Institute  
Department of Science and Technology  
Gen. Santos Ave., Bicutan, Tagig, Metro Manila*

## ABSTRACT

Three communal food processing centers were established in the regions, which are strategically located in the Philippines, namely, Regions 2, 5, and 12. The available raw materials in each region were matched with appropriate technologies, which were the bases of equipment purchase. Training of manpower to operate the centers, as well as technology adoptors, was conducted by FNRI personnel. Commercialization of FNRI technologies in the regions is instrumental in attaining the goals of both economic and nutritional improvement. Recommendations in the efficient operation of the centers were formulated based on the problems encountered during the implementation of the project.

## INTRODUCTION

An effective and efficient mechanism for the transfer and commercialization of FNRI technologies is by way of creating communal food processing centers in the regions. The concept focuses on making common food processing facilities available right where the materials are (Teller, 1991). The strategy has the advantages of: (1) minimizing price mark-up usually arising from the use of a middleman, in purchasing raw materials; (2) simplifying collection, distribution and transport problems; (3) decreasing food losses due to seasonality, by immediately processing the crops; (4) creating livelihood and employment opportunities in the regions, and (5) providing nutritional foods for those who need them most.

The establishment of communal centers in the regions provides start-up businesses in new technology ventures with a center equipped for production, and enables businessmen to get established and operate on their own within the shortest possible time. Entrepreneurs benefit from results of research and development output, reduced overhead expenses and marketing assistance and promotions provided by the Department of Science and Technology (DOST).

Entrepreneurship has been recognized as the engine of economic change in practically all parts of the world (Anonymous, 1994). Therefore, efforts at upgrading the economic conditions of Filipinos must include development of entrepreneurship as a significant tool of their empowerment.

The DOST recognized that its role in nurturing a science and technology-based entrepreneurship is major and crucial in advancing common efforts towards Philippines 2000. Thus, establishment of communal food processing cen-

ters in the regions not only enhances a series of economic activities, but also aims at a goal of providing consumers with nutritional foods produced from indigenous sources. Improvement of nutrition will also create a healthy citizenry, an important role in the human resource development of the country.

The objectives of this project are the following:

1. To establish communal food processing centers in Regions 2, 5 and 12;
2. To train staff members to man the operations of the centers;
3. To train and develop entrepreneurs and mothers' groups on technology-based business ventures, thus spurring a chain of economic activities in the regions;
4. To provide livelihood and employment opportunities ranging from producers of raw materials (farmers) to suppliers and distributors of food products; and
5. To make available nutritional food products in the regions, and thus, to showcase the commercial viability of FNRI technologies.

## METHODOLOGY

### Regional Sites

The project was made possible with efforts coming from four implementors, namely, (1) Technology Application and Promotions Institute (TAPI), which provided the financial requirements for major equipment and initial maintenance and operating expenses; (2) Food and Nutrition Research Institute (FNRI), which organized the project, conducted assessment of the area where the center is built, provided sources of technologies, and planned for the layout of the center, trained manpower to run the center, as well as technology adoptors, and monitored quality of products; (3) DOST Regional Office, which identified and assessed the resources and capabilities available in the region and saw to the efficient implementation of the project, and (4) State Colleges and Universities (SCUs) and other technology users which constructed/renovated space for the center; assigned capable and trainable staff to man the center, implemented the project and prepared productin and sales reports.

The choice of the region where the Center is built depends on the availability of raw materials for food processing, potential markets for products, willingness of the DOST regional office to implement the project and commitment of the SCUs in identifying qualified manpower and capability in efficiently running the center. FNRI conducted consultative meetings and identified three regions strategically located in the Philippines, namely, Region 12 in Mindanao, Region 2 in Northern Luzon and Region 5 in Southern Luzon, based on the above, criteria.

The first center established was in Region 12, which is housed in the DOST Regional Office in Cotabato City. It was later transferred to Sultan Kudarat Poly-

technic State College (SKPSC) in Isulan, Cotabato. The second center is built in Region 2, and is housed at the Isabela State University (ISU) in Echague, Isabela. Lastly, the third center is at the Bicol College of Arts and Trades (BCAT) in Naga City, Camarines Sur.

Appropriate technologies were matched with identified raw materials. Based on the technologies, the equipment were determined and purchased, and later were shipped, installed and tested in the regions by the FNRI.

### **Training of Equipment Operators**

The equipment operator is prescreened or interviewed by the SCUs managing the communal center. This is to ensure that the user's operator is knowledgeable in the proper operation of the machine and is aware of the required safety practice.

FNRI food technologists and engineers are responsible in the proper training of all staff of the communal centers. Products and processes are demonstrated by FNRI staff and practised by the designated operators, until such time that the operator can run the facilities on his own.

Product quality control was also emphasized and each step in the operations is well-explained in order to avoid rejects. Sanitation practices, plant hygiene and environment-friendly waste disposal systems are also taught.

### **Training of Technology Adoptors and Entrepreneurs**

Seminar-workshops and trainings are conducted to teach entrepreneurs on in-plant operations on the technologies selected for each center. Organized by the plant manager, the clientele includes farmer's cooperatives, prospective entrepreneurs, businessmen, mothers' groups and institutional market representatives like DOH, DSWD, NNC, DECS, religious organizations involved in feeding center operations, and others who are interested in starting their own food business enterprise.

FNRI food technologists handle the food demonstrations and trainings. Participants avail of hands-on experience and practice on the processing of nutritional food products. Emphasis on the nutritional component of the training is given, in order to have competitive advantage of the products over existing commercial products of the same category.

An introduction on how to run the business, cost of food production, pricing of products, and profitability of the enterprise is taken care of by FNRI food engineers. The trainee gets acquainted with the machines and simple business operation and is assured of the availability of the equipment in the locality.

### **Socio-Economic Aspects**

Technology adoptors and users of the communal center are identified and

screened by the Plant Manager. Service fees are charged for the use of the equipment. The manager sees to it that facilities are operated only by trained personnel and not by the entrepreneurs alone by themselves. Status of technology development, business operation, budgetary requirements, and annual gross income are reported by the businessmen to the Plant Manager. The businessmen can build their market while using facilities of the center, and little by little start on their own, after having established their markets.

With advice from the FNRI and TAPI, the communal center operator may price the common service facilities to include the following lease rates: (1) depreciation costs, (2) tool costs (3) repair and maintenance costs, (4) power cost, (5) overhead cost (6) profit margin, and (7) value added tax.

Benefits that could be derived from such business activities could create better market for farmers' produce, employment opportunities for factory workers and product distributors and income generation in the locality. Moreover, the availability of nutritional food products in the area is instrumental in the improvement of the nutritional status of the community (De Guzman. 1991).

## **RESULTS AND DISCUSSION**

### **Communal Center in Region 12**

A consultative meeting with the DOST 12 management, together with the group composed of intergovernment agencies and non-government organizations (NGOs) in May, 1991 was initiated by FNRI. The activity was done to generate awareness of the purpose of the undertaking, to establish rapport and solicit support and cooperation among the people in the region, identify available resources and facilities, and to assess needs with regards to materials, market, place, entrepreneurs' interest in food business and other relevant concerns.

The abundance of squash and fish as raw materials were the basis of determining the appropriate technologies for the center. The following food technologies and equipment facilities were presented and installed:

#### **Appropriate Technologies**

- o Squash Flour
- o Squash Ketchup
- o Squash Chips
- o Squash Nutrient Cubes
- o Squash Noodles
- o Squash Bakery Items
- o Fish Protein Concentrate
- o FPC Noodles
- o FPC Kroepeck
- o Sardines
- o FPC Bouillon Cubes

#### o Squid Powder Palabok Mix

The equipment were installed at the center by FNRI food engineers and test runs were conducted. The operator of the equipment who was assigned by the center management was rigidly trained on the different processes and proper use and maintenance of equipment. Complete product information on the above technologies are left to the manager of the center.

The center was inaugurated on December 11, 1991, which was attended by a large group comprised of the business sector and representatives of GOs and NGOs in Cotabato City. The large turnout of participants was due to a media coverage by a radio interview of two FNRI staff prior to the inauguration.

Seminar-workshops were conducted and were attended by several participants,

After the seminar-workshops, those who signified their interest to go into food business were identified. In Region 12, these included: (a) village of 200 resettled families which operate and run their own cooperative, were interested in the production of supplementary foods for their feeding centers; (b) restaurant owners wanted to produce squash ketchup and bakery products with squash; (c) other groups wanted to produce fish products.

One year after the installation of the equipment. DOST 12 management was not able to run the center as expected. Interest in the food business dwindled due to lack of capability of DOST 12 to open the facilities to the public, despite good prospects during the initial stage.

The center was transferred to Sultan Kudarat Polytechnic State College in Isulan, Cotabato, also part of Region 12. Under new management, which FNRI trained anew, the center was continually producing flours from squash, yam, rootcrops and cereals. As of July 1994, reports revealed that the flours made from the center have prospects of being exported to Japan.

From these intermediate products like flours, final products are also made like doughnuts, cookies, polvoron and other bakery items which seems to be salable among the students and in the nearby community stores. The activities are being sustained up to the present.

Monitoring of plant activities and product quality are being undertaken by FNRI personnel periodically. New research and development technologies are continually fed to the center for expansion of their operations. Upgrading of facilities is being negotiated with TAPI.

### **Communal Center in Region 2**

The second center established by FNRI was at the ISU, in Echague, Isabela on December 13, 1992. Management of the center was delegated to ISU faculty

of Food Engineering staff, since they are the right people to operate the machines.

Peanut and other legumes, as well as cereals, were identified as the abundant raw materials in the area. Isabela was chosen as the site primarily due to being the peanut center in the Philippines, and secondarily due to its strategic location in the region as a central province from Cagayan in the North to Nueva Viscaya and Quirino in the South. It has also an airport at Cauayan town, so it is accessible by air and land transportation.

The appropriate technologies and equipment installed at the center in Region 2 are the following:

#### **Appropriate Technology**

- o Peanut Butter
- o Peanut Brittle
- o Coated Peanut
- o Peanut Noodles
- o Banana-Peanut Blend
- o Rice-mongo Crunchies
- o Rice-soy Noodles
- o Cereal-Legume Blends
- o Squash Products
- o Cereal Flours
- o Legume Flours
- o Rootcrops Flours

#### **Equipment Installed**

- o Peanut Roaster
- o Flour Mill
- o Dryer
- o Emulsifier/Grinder
- o Deep Fryer
- o Dough Mixer
- o Coating Machine
- o Blender
- o Cooking Facilities
  - o Noodle machine
- o Sealers
- o Weighing Scale

Seminar-workshop on the different technologies were conducted and 72 participants were trained at the center as shown in the following table:

<b>Date</b>	<b>Food Product</b>	<b>Venue</b>	<b>No. of Participants</b>
3-28-92	<ul style="list-style-type: none"> <li>o Squash Ketsup</li> <li>o Peanut Butter</li> <li>o Wheat-Soy Noodles</li> <li>o Rice-Mongo Crunchies</li> <li>o Fruit Cocktail</li> </ul>	ISU, Echague, Isabela	30
6-26-92	<ul style="list-style-type: none"> <li>o Cereal/Rootcrop/Legume Flours</li> <li>o Nutritious Noodles</li> </ul>	ISU, Echague	42

o Cereal-Legume  
Snackfoods

Total: 72

The participants represented all the provinces of Region II including businessmen in Isabela. Farmers cooperatives, ISU teachers and other staff, and prospective entrepreneurs.

The ISU management and DOST Region II Director have given full support for this project. DOST management gave an initial P50,000.00 for capital in operating the center.

Six months after its establishment, the center was producing several products like flour products, different flours (cassava, rice/corn, rootcrops, legumes), cereal-legum snack-foods. The center has expanded its operations into meat products, since the area is also noted for livestock (cattle, swine, poultry) production.

The next table shows the gross sales of products of the center:

Items	Gross Sales (P), 1993			
	July	August	September	October
Tapa, pork	202.50	1,305.00	1,170.00	2,160.00
Tapa, beef	275.00	1,045.00	1,430.00	385.00
Embotido	2,800.00	4,400.00	3,475.00	1,775.00
Skinless longanisa	630.00	4,185.00	4,995.00	2,655.00
Tocino	225.00	2,205.00	1,980.00	2,160.00
Chicharon	520.00	1,640.00	1,380.00	1,010.00
Doughnut	416.00	1,312.00	-	-
Polvoron	432.00	882.00	2,232.00	1,503.00
<b>TOTAL:</b>	<b>5,500.00</b>	<b>16,974.00</b>	<b>16,662.00</b>	<b>12,232.00</b>

Other products which are being tested in the market are snackfoods from rice-mongo, cassava and siopao.

The center is also catering to flour milling of bulgur, green peas, rice and corn, which are used by different clientele in the area including BIDANI, cooperative members and other entrepreneurs involved in the baking business. The center has employed two personnel on a full-time basis. Moreover, they have started paying out for the monthly installment of their equipment to TAPI.

### Communal Center in Region V

The center was established at BCAT, Naga City in December, 1992. Region V is the rice center in Southern Luzon, and because of this, processing of the commodity into extruded snackfoods and weaning foods is appropriate. The identified technologies and equipment installed are as follows:

## Appropriate Technology:

- o Instant Rice-Mongo Baby Food
- o Rice-Mongo Curls
- o Corn-Mongo Curls
- o Instant Corn-Mongo Baby Food
- o Other Products

## Equipment Installed:

- o Extruder Cooker
- o Dryer with trays
- o Coating Machine/Mixer
- o Cooking Facilities
- o Packaging Facilities
- o Grinder with Emulsifier  
Blade
- o Weighing Scale

Training of personnel to run the center was done very rigidly. Seminar-workshops on extruded products and other technologies were conducted also as follows:

Although the response during the negotiations and seminar-workshops conducted were favorable, the full operation of the center was never attained. After the delivery of equipment to Region V, minor problems due to transport, occurred. After test runs, the barrel and screw have worn-out. Replacement was made by the manufacturer, and even when everything was in running condition as confirmed by engineers of the Metals Industry Research and Development Institute of DOST, the center have not gone into operation.

It turned out that there was lack of efficient management of the center and also, lack of support from BCAT officials.

The equipment were returned to TAPI, through FNRI, after two years due to non-operation of the center.

### Socio-Economic Component

FNRI experiences in technology transfer over the years have shown that the food products were very useful in catering to the social market as in feeding programs, as an alternative to the foreign food donations. Among the beneficiaries of these feeding programs were: (1) San Juan, Metro Manila in 1986; (2) Negros Occidental in 1987; (3) Tangos, Navotas in 1988; (4) Cogeo, Antipolo in 1989; (5) Quezon City Elementary Schools in 1990; and (6) Mt. Pinatubo evacuees in Pampanga in 1991. (FNRI-NPDS Annual Reports). Due to the phasing out of donated food used in the food assistance component of the Philippine Nutrition Program, the utilization of indigenous raw materials was, therefore, necessary.

Being principally a research institution, FNRI does not have the capability to produce developed supplementary foods in commercial quantities. There is, thus, a need to transfer the technologies to entrepreneurs for commercialization, to make it readily available to its target clientele.

Strategies used in effecting commercialization of technologies is the creation of communal processing centers in the regions, as well as common service facilities located nationwide. This strategy bridges the wide gap between re-

search and its utilization, in order to make research findings a viable contribution to the economic situation and push for Philippines 2000, when Filipinos are adequately provided in both nutritional and economical aspects.

### **Monitoring and Evaluation**

1. The reason why Region XII, at first, could not really go on with the smooth running of the plant was because the top management did not give full attention to the project. It may not be the entire responsibility of the management, though, it may also be due to lack of capital or financial capability of entrepreneurs to go into business. Aside from budget constraints, market demands may not have been fully studied.

2. Transfer of the center to SKPSC has made possible for the center to operate due to presence of trainable manpower to run the machinery and equipment; student population served as the market of the products; top management support was fully given.

3. In ISU, financial support was extended by DOST 02 management as starting capital; student population is a captive market; top management of ISU was very supportive of the project; presence of cooperatives and involvement is very strong and working in region 2. Improvement of packaging materials for their products is an immediate concern and needs technical and financial assistance.

4. The center in Region 5 never worked because it did not have initial resources to provide financial and management support. The extension person who was very enthusiastic at first, was transferred to another office and since then support was not given to the center.

### **CONCLUSIONS AND RECOMMENDATIONS**

In order to provide small starting entrepreneurs new technology ventures, the FNRI has established three communal food processing centers in Regions 02, 05, and 12. Despite the presence of equipment facilities, obstacles in venturing into micro or small scale production are: (1) lack of skills; (2) budget constraints; (3) market demands (4) packaging materials (5) quality control problems, and (6) full support from the management of the centers.

It is, therefore, recommended that the provision of equipment facilities and technology resources should also be accompanied by the following components: (1) raw material resource linker, (2) financing resource linker (3) market resource linker, (4) promotions and communications resource linkers and possibly a seminar on how to handle and improve small business.

## REFERENCES

1. Teller, Ph. 1991. Food processing in farmers' hands, a motor for an increase in agricultural production. *Appropriate Technology Source*, Vol. 19, No. 2, p. 2.
2. Anonymous, 1994. *The Facilitator's Hand Book for Entrepreneurship Development Among Rural Women*. United Nations Asian and Pacific Centre for Transfer of Technology, p1.
3. Ge Guzman, Ma. P.E.; Marero, L.M.; Garcia, E.I.; Martin, A.M. Jr. and Molano, B.T. 1992. Prospects for economic productivity and nutrition intervention through communal food processing centers. *Philippine Journal of Food Science and Technology* 16 (2): 17-22.
4. Annual Reports of the Nutritional Products Development Section, Food and Nutrition Research Institute-DOST, Bicutan, Tagig, Metro Manila. 1986 - 1991.

### **Equipment Installed**

- o Dryer
- o Steamer
- o Cooking facilities
- o Noodle machine
- o Freezer
- o Flour Mill
- o Canning facilities
- o Packaging facilities
- o Food Blender

### **Product**

- o Banana Soybean Blend
- o Banana Peanut Blend
- o Rice Mongo Crunchies
- o Rice Mongo Noodles
- o Squash Flour
- o Sardines

**Product**

- o Banana Peanut Blend
- o Rice Mongo Sesame Blend
- o Rice Mongo Crunchies
- o Squash Chips
- o Fish Noodles
- o Rice Mongo Noodles
- o Peanut Butter
- USM, Kabacan
- Cotabato
- o Banana Soy Blend
- o Corn Mongo Kroepeck
- o Rice Mongo Sesame Blend
- o Squash Noodle
- o Seaweed Noodle

Total No. of Participants Trained 114



# EVAPORATIVE COOLING STORAGE OF TOMATO AND SWEET PEPPER FRUITS HARVESTED AT DIFFERENT STAGES OF RIPENESS

A. L. Acedo Jr., F. G. Enriquez and M. A. Mante  
Postharvest Technology Laboratory, Dept. of Horticulture  
Visayas State College of Agriculture  
Baybay, Leyte 6521-A, Philippines

## ABSTRACT

Fresh tomato and sweet pepper fruits harvested mature-green (peel color stage 1 or CI 1), breaker (CI 2), turning (CI 3) and more red-than-green (CI 4) lasted for about 8-14 days at ambient (28.0-31.5°C; 75-88% relative humidity). Storage in simple evaporative coolers (EC) (25-28°C; > 90 RH) markedly prolonged the postharvest life of CI 1-2 tomatoes by 8-10 days and approximately doubled the postharvest life of CI 1-4 sweet peppers due mainly to the inhibition of fruit shriveling. Storage in the EC also reduced fruit weight loss considerably. The CI 3-4 tomatoes had reduced weight loss and shriveling but showed early disease symptoms. Fruit ripening was not delayed by EC storage, except in sweet peppers harvested at CI 1 most of which attained full ripe stage were comparable among EC-stored and ambient-stored fruits. The sensory quality of the ripe fruits did not vary also but the overall flavor of EC-stored sweet peppers was rated better than that of fruits stored at ambient.

## INTRODUCTION

Evaporative cooling system creates a cool and humid environment of water. The decrease in temperature is small but the increase in humidity is substantial that postharvest quality deterioration of fresh produce caused particularly by moisture loss can be effectively controlled. Several inexpensive evaporative cooling techniques have been tried as alternatives to the high-cost refrigerated storage method (3, 5, 6).

In a recent study, a simple box-type evaporative cooler (EC) was developed and found to increase the relative humidity by 10-20% and decrease the temperature by 1-6°C relative to ambient (1). Mature-green tomato (*Lycopersicon esculentum* Mill.) and sweet pepper (*Capsicum annuum* L.) fruits stored in the EC had a markedly reduced weight loss, shriveled only slightly and as a result, had a postharvest life twice stored at ambient (2). Ripening of the fruits differed. In tomato which is a climacteric fruit, ripening was not retarded considerably but the fruits developed deep red peel. On the other hand, sweet pepper, a non-climacteric fruit, showed normal but much delayed ripening changes in contrast to that of ambient-stored fruits, most of which failed to ripen fully before reaching

the end of their postharvest life.

Tomatoes and sweet peppers are often harvested when ripening changes become visible. A series of experiments was then conducted to determine the postharvest life of tomato and sweet pepper fruits harvested and stored in the EC at various degrees of ripeness. The physicochemical and sensory quality attributes of the fruits were also obtained.

## MATERIAL AND METHODS

Freshly harvested 'Improved Pope' tomato and 'Grossum' sweet pepper fruits at the mature-green, breaker, turning, and more red-than-green stages of ripeness were secured from local vegetable growers. Thirty (30) uniformly sized and defect-free fruits were used for each treatment.

The box-type EC developed earlier (1) was used. Before storage of the fruits, the EC was washed with detergent and 1% sodium hypochlorite solution prepared using the commercial bleach. One EC was used for each stage of ripeness. Storage under ordinary room condition (ambient) was included for comparison.

During storage, the temperature and RH were monitored using either a Tri-sense electronic temperature-RH meter or improvised dry-bulb and wet bulb thermometers. The vapor pressure deficit (VPD) was traced from a psychrometric chart using the temperature and/or Rh readings.

Peel color development of the fruits was visually scored using a color index (CI) of 1-6 in which CI 1 - mature-green, CI 2 - breaker, first trace of red or orange, CI 3 - turning, more green than red or orange, CI 4 - more red or orange than green, CI 5 - red with traces of green, and CI 6 - full red. The number of days for each fruit to reach CI 6 was taken as a measure of the ripening period.

At the ripe stage (CI 6), the total soluble solids (TSS), titratable acidity (TA), pH, and sensory quality were determined. From a combined blended sample of 3 fruits TSS was read with an Atago hand refractometer while TA was analyzed by the titrimetric method with standardized 0.1N NaOH and using 1% phenolphthalein as indicator. pH was measured using a Hanna electronic pH meter. The sensory quality attributes (pulp color, mouth feel, aroma, and overall flavor) of raw tomatoes and blanched sweet peppers were evaluated by 10-15 trained panelists on a 10-cm line, with 0 denoting as poor and 10 as excellent characteristic.

Measurement of fruit weight loss, degree of shriveling and decay incidence were also taken. Weight loss was expressed in percentage of the initial fruit weight while the degree of shriveling was scored using an index of 1, 2, 3 and 4 for none, slight moderate and severe shriveling, respectively. Decay incidence was determined by taking the number of disease-infected fruits which was then expressed in percentage of the total number of fruit samples.

The potential postharvest life was estimated as the number of days from the start of storage up to the date when the fruits had moderate degree of shriveling (score of 3) or just before the fruits developed disease symptoms or became overripe.

The results presented are pooled means for 2-3 storage trials.

## RESULTS AND DISCUSSIONS

**Storage conditions.** Low temperature and very high RH were estimated established in the EC throughout the storage period of tomato and sweet pepper fruits (Figure 1). The temperature was 1-4°C and 1.5-5.8°C lower than the ambient storage temperature for tomatoes (Figure 1A) and sweet peppers (Figure 1D), respectively. The RH in the EC was maintained at 95% or higher (Figure 1B) while the ambient RH fluctuated and ranged from 75-88% (Figure 1E). As a consequence, the VPD decreased from 3.2-7.7 mb during ambient storage of tomato (Figure 1C) or 5.8-10.5 mb during ambient storage of sweet pepper (Figure 1F) to about 0.5-1.5 mb in the EC. On the average, the VPD in the EC was about 1 mb while the ambient VPD during storage of tomatoes and sweet peppers, 5.4 mb and 7.1 mb, respectively (Table 1). These VPD values suggest that the ambient storage atmosphere for fruits five times drier and may therefore dry out the fruits five times faster than the EC atmosphere. The ambient storage environment for sweet peppers was much drier, about seven times greater than that in the EC. This was due to the higher temperature and lower RH during sweet pepper storage than that during tomato storage (Figure 1; Table 1).

**Fruit ripening changes.** EC storage did not delay peel color development of tomatoes (Figure 2). It even promoted ripening of fruits harvested and stored when turning (CI 3). The latter fruits fully ripened after 4.8 days of storage which was significantly earlier than that of the same fruits stored at ambient (6.9 days) (Table 2). The other EC-stored fruits had ripening periods comparable to that of ambient-stored fruits, ranging from 9-11 days, 7-8 days, and 3-5 days for fruits harvested and stored when at CI 1, 2 and 4, respectively.

The same trend in peel color changes was obtained in sweet peppers, except for fruits harvested mature-green (CI 1) (Figure 3; Table 2). More than 50% of the CI 1 fruits stored at ambient failed to reach full ripe stage and those that fully ripened did so after 9 days of storage or about one week earlier than that of EC-stored fruits. This response is similar to that observed in an earlier study (2). Sweet pepper, being a non-climacteric fruit, does not exhibit dramatic ripening changes once initiated to ripen. When harvested green and stored at ambient, the fruits became severely shriveled and unmarketable before attainment of full red peel color.

The TSS, TA and pH at the red-ripe stage of tomatoes and sweet peppers harvested at various degrees of ripeness and stored at ambient or in the EC were statistically comparable (Table 2). The ripe tomatoes had a TSS ranging from 3.6-4.9%, TA from 0.26-0.34% citric acid, and pH from 5.6-5.8. The TSS:TA ratio was consistently > 10. These results show that ambient and EC storage did not adversely affect the compositional quality of the fruits. High quality tomatoes are characterized by containing > 3% soluble solids and 0.32% TA, and having a TSS:TA ratio of > 10 (4). On the other hand, the TSS, TA and pH of ripe sweet peppers ranged from 2.3-3.1%, 0.28-0.38%, and 6.4-6.8, respectively. The TSS:TA

ratio was about 10 or lower.

No significant differences in sensory quality of ripe tomatoes were obtained (Table 3). All sensory attributes were rated 6.6 and higher, indicating that the eating quality remains acceptable regardless of whether the fruits are harvested and stored in the EC or at ambient when at CI 1-4. Similarly, rating difference for pulp color (6.7-8.1), texture or mouth feel (7.1-7.3), and aroma (6.5-7.3) of ripe sweet peppers were not significant. However, the EC-stored fruits developed a more prominent pepper taste and juicy texture, resulting in better overall flavor (6.6-6.8) than that of ambient-stored fruits (4.8-5.8).

**Fruit weight loss.** EC storage reduced weight loss of tomatoes and sweet peppers considerably (Figure 4). Ripeness stage at harvest did not seem to affect the magnitude of weight loss. In tomatoes, weight loss was only slightly higher in CI 3-4 fruits than in CI 1-2 fruits (Figure 4A). In sweet peppers, the CI 1-3 fruits had similar weight loss which was slightly lower than that of CI 4 fruits (Figure 4C). In general, all EC-stored fruits lost less than 10% of their initial weight at the end of storage.

At ambient where a drier storage environment prevailed, both tomatoes and sweet peppers exhibited rapid weight loss and at the end of storage, lost about 14-17% and 34-41% of their initial weight, respectively (Figure 4B, 4D). The stage of ripeness at harvest did not effect wide variations in weight loss. The extremely higher weight loss of ambient-stored sweet peppers can be attributed to the much drier storage environment as compared to that during tomato storage. In addition, tomatoes are waxy and have thick cuticle which serves as resistance factor to transpirational water loss.

Tomatoes and sweet peppers are normally traded by weight basis so that any loss in weight represents a financial loss. The present study demonstrates that losses in saleable weight of the fruits regardless of harvest maturity can be minimized by EC storage.

**Fruit shriveling.** Shriveling is the primary cause of deterioration in physical quality of tomatoes and sweet peppers stored under ordinary tropical condition. This fact is again illustrated in Figure 5B and 5D which show the rapid development of shriveling in ambient-stored fruits. Tomatoes harvested at CI 3-4 had a greater degree of shriveling than fruits harvested at CI 1-2 (Figure 5B). Shriveling of sweet peppers was comparable among fruits harvested green (CI 1) or ripe (CI 2-4) (Figure 5D).

When the fruits were stored in the EC, shriveling was totally inhibited in tomatoes (Figure 5A) and was hardly noticeable in sweet peppers (Figure 5C). Furthermore, it can be retracted from Figure 4 that all EC-stored fruits lost less than 10% of their initial weight. This result together with that shown in Figure 5 suggest that shriveling symptoms in 'Improved Pope' tomatoes and 'Grossum' sweet peppers become conspicuous only when the fruits lose at least 10% of their initial weight.

**Postharvest life.** EC storage markedly prolonged the postharvest life of tomatoes when harvested at CI 1 and CI 2 by ambient, respectively (Figure 6). The EC-stored fruits harvested at CI 3 had also a significantly longer postharvest life but the difference relative to that of ambient-stored fruits was only 2.5 days. The fruits harvested at CI 4 had comparable postharvest life under ambient or EC condition. On the other hand, sweet peppers harvested and stored in the EC when at CI 1, 2, 3, and 4 lasted for 21.7, 17.3, 16.7 and 15.7 days, respectively, or about twice longer than the postharvest life of fruits stored at ambient (8.3-9.6 days).

Postharvest life extension by EC storage was due primarily to the inhibition of fruit shriveling. The resultant maintenance of turgidity translated into a longer ripe life of the fruits. As storage progressed, the ripe EC-stored fruits became too soft and overripe. In addition, disease infection by the soft rot pathogen (*Erwinia carotovora*) also set in and was more serious in tomatoes (Figure 7A-B) than in sweet peppers (Figure C-D). It affected early the fruits harvested at CI 3-4. Because of this and in spite of the inhibition of shriveling, the CI 3-4 tomatoes did not last a lot longer than those ambient-stored. During ambient storage, a much reduced decay incidence was observed but in tomatoes, disease development accounted partly for the short shelf life of the fruits.

The findings of the study establish the great potential of the EC as an effective storage medium. For tomatoes and perhaps other climacteric fruits, the use of the EC to prolong postharvest life may only be feasible for fruits at the mature-green to breaker stages of harvest, the EC can be used as a temporary storage to prevent shriveling and excessive loss in saleable weight. On the other hand, for sweet peppers and perhaps other non-climacteric fruits, the EC is suitable for the storage of fruits when harvested at the mature-green and ripening stages.

### ACKNOWLEDGMENT

The financial support from the International Foundation for Science partly made this study possible.

### REFERENCES

1. Acedo, A. L. Jr. 1993. Development of improved evaporative cooling technique as low-cost storage for fresh vegetables, in *Towards More Effective Utilization of Resources for Sustained Development*, Proceedings of 1st IFS-NRCP Seminar- Workshop, 23-29 April 1993, Manila, Philippines (Published by IFS, Sweden, 243-255).
2. Acedo, A. L. Jr. 1993. Effects of evaporative cooling conditions on the postharvest life of tomato and sweet pepper fruits. *Philippine Journal of Crop Sciences* 18: 37.
3. Garcia, J. L. and O. K. Bautista. 1984. Ricehull ash: A potential storage medium for small farmers. *Appropriate Postharvest Technology* 1: 4-5.

4. Kader, A. A., L. L. Morris, M. A. Stevens and M. Albright- Holton. 1978. Composition and flavor quality of fresh market tomatoes as influenced by some postharvest handling procedures. *Journal of the American Society for Horticultural Science* 103. 6-13.
5. Labios, E. V. and O. K. Bautista. 1984. Moisture loss in pechay stored in evaporative coolers. *Postharvest Research Notes* 1. 28.
6. Redulla, C. A., M. U. Reyes and S. C. Andales. 1984. Temperature and relative humidity in two types of evaporative cooler. *Postharvest Research Notes* 1. 25-27.

**Table 1.** Average temperature, relative humidity (RH) and vapor pressure deficit (VPD) during the storage of tomato and sweet pepper fruits at ambient and EC condition.

Storage Condition	Temperature <sup>1</sup> (°C)	RH <sup>1</sup> (%)	VPD <sup>1</sup> (mb)
<b>Tomato storage</b>			
EC	25.9 ± 0.9	96.5 ± 1.8	1.1 ± 0.5
Ambient	28.4 ± 1.0	85.6 ± 5.2	5.4 ± 2.1
<b>Sweet pepper storage</b>			
EC	26.4 ± 1.4	97.5 ± 1.9	0.8 ± 0.6
Ambient	29.3 ± 1.7	82.9 ± 6.2	7.1 ± 3.0

<sup>1</sup>Mean ±SE.

**Table 2. Ripening period and chemical attributes at the ripe stage of EC- and ambient-stored tomato and sweet pepper fruits harvested at different stages of ripeness (CI 1-4).**

Treatments		Days to Full Ripe	% Ripe Fruits	TSS %	TA % citrate	pH
<b>Tomato fruits</b>						
EC	CI 1	10.4a	94.4	4.1	0.34	5.7
	CI 2	7.3b	96.6	4.3	0.34	5.7
	CI 3	4.8c	84.7	3.6	0.30	5.6
	CI 4	3.6c	88.1	3.8	0.28	5.7
Ambient	CI 1	9.4a	94.5	3.9	0.29	5.6
	CI 2	7.2b	92.9	4.9	0.30	5.7
	CI 3	6.9b	85.9	4.7	0.28	5.8
	CI 4	3.7c	100.0	4.0	0.30	5.8
<b>Sweet pepper fruits</b>						
EC	CI 1	16.4a	96.6a	2.4	0.31	6.4
	CI 2	8.8b	100.0a	2.3	0.28	6.4
	CI 3	5.9c	100.0a	2.6	0.33	6.4
	CI 4	3.7d	100.0a	2.9	0.38	6.4
Ambient	CI 1	9.1b	42.2b	3.0	0.32	6.8
	CI 2	8.3b	88.9a	3.1	0.28	6.7
	CI 3	5.2c	100.0a	3.1	0.31	6.8
	CI 4	3.6d	100.0a	3.0	0.28	6.8

Mean separation within columns per commodity by DMRT, 5%.

**Table 3. Sensory quality at the ripe stage of EC- and ambient-stored tomato and sweet pepper fruits harvested at different stages of ripeness (CI 1-4).**

Treatment		Pulp Color	Mouth Feel	Aroma	Overall Flavor
<b>Tomato fruits</b>					
EC	CI 1	7.2	7.6	7.0	7.5
	CI 2	7.8	7.3	7.5	7.6
	CI 3	7.8	7.7	6.8	7.4
	CI 4	8.0	7.6	7.6	7.4
Ambient	CI 1	7.3	7.6	7.2	7.8
	CI 2	6.6	7.8	7.2	8.0
	CI 3	7.5	7.6	6.9	7.4
	CI 4	8.1	8.0	8.0	8.1
<b>Sweet pepper fruits</b>					
EC	CI 1	6.7	7.3	6.8	6.8a
	CI 2	3.1	7.1	7.2	6.8a
	CI 3	7.0	7.3	7.1	6.7a
	CI 4	7.6	7.3	7.3	6.6a
Ambient	CI 1	6.5	6.7	6.5	4.8b
	CI 2	7.2	6.7	6.6	5.3b
	CI 3	7.9	6.6	7.3	5.2b
	CI 4	7.6	7.6	6.9	5.8ab

Mean separation within columns per commodity by DMRT, 5%. Each sensory attribute was rated on a 10-cm line, with 0 denoting as poor and 10, as excellent characteristic.

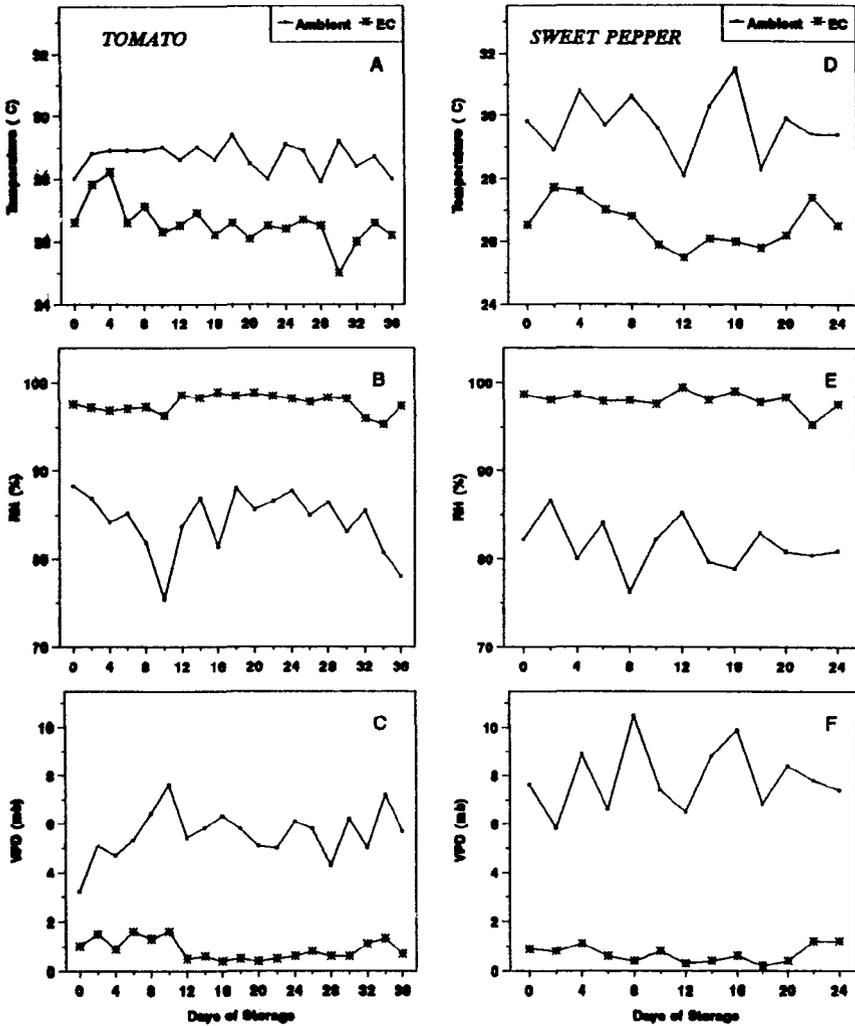


Figure 1. Temperature, RH and VPD during storage of tomato (A,B,C) and sweet pepper (D,E,F) fruits at ambient and EC condition.

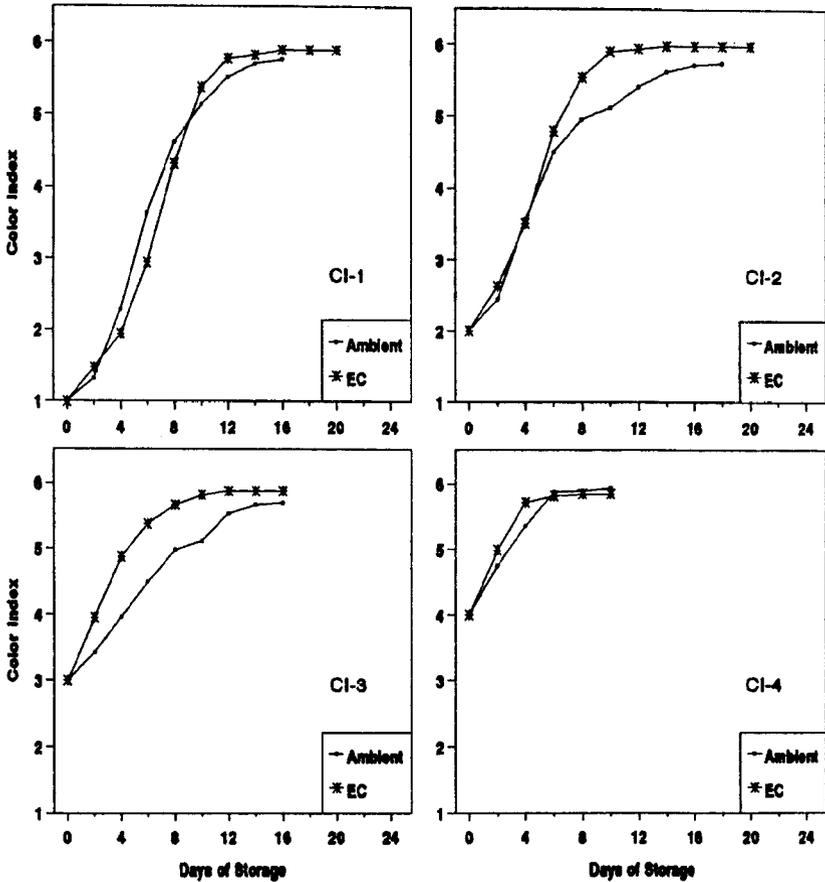


Figure 2. Peel color development of EC- and ambient-stored tomato fruits harvested at different stages of ripeness (CI 1-4).  
*(Peel Color Index, CI: 1-green mature; 2-breaker, first trace of red or orange; 3-turning, more green than red or orange; 4-more red or orange than green; 5-red with traces of red or orange; 6-full red)*

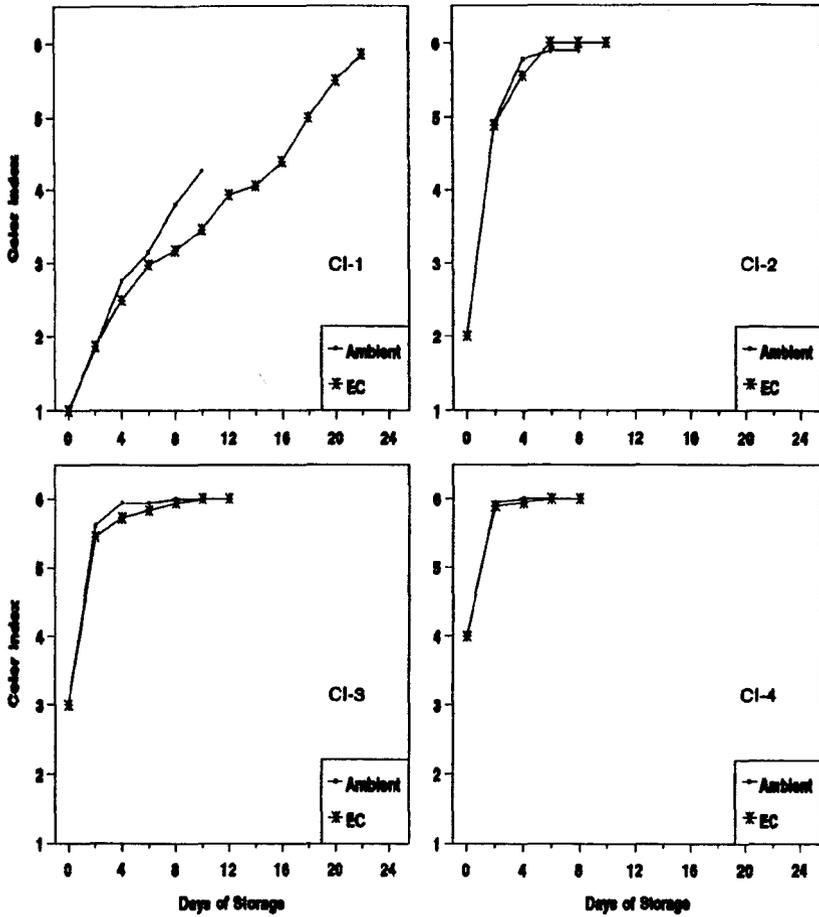


Figure 3. Peel color development of EC- and ambient-stored sweet pepper fruits harvested at different stages of ripeness (CI 1-4). (see notes in Figure 2)

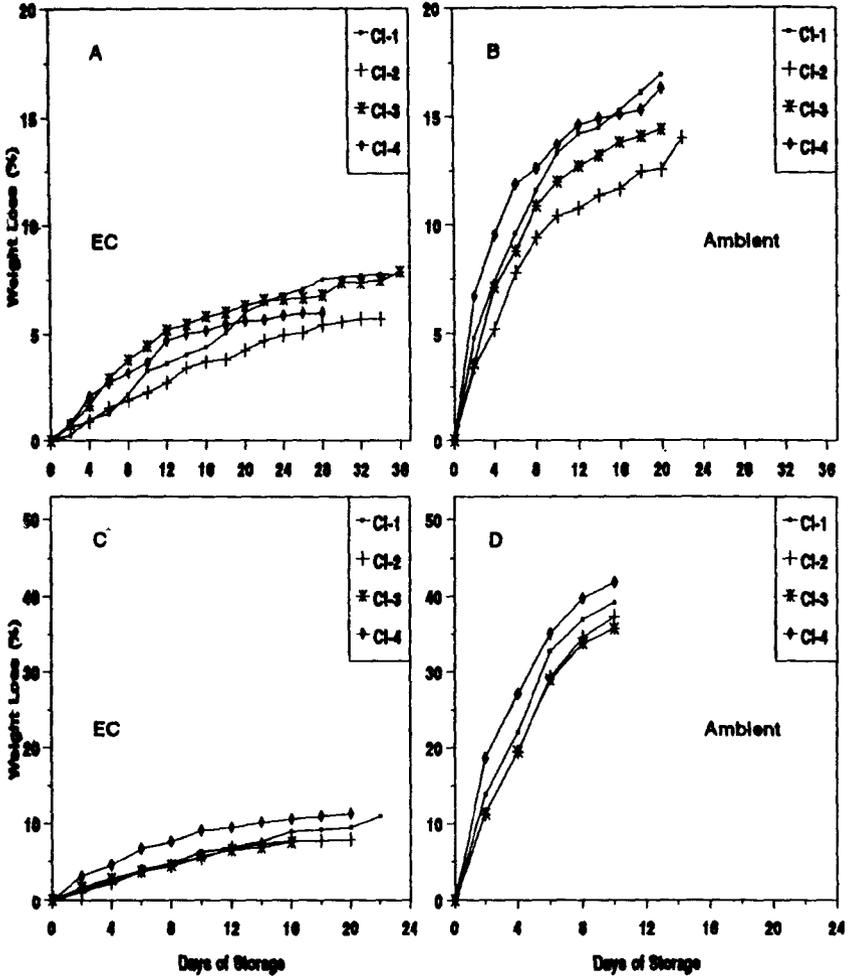


Figure 4. Cumulative weight loss of EC- and ambient-stored tomato (A,B) and sweet pepper (C,D) fruits harvested at different stages of ripeness (Cl 1-4).

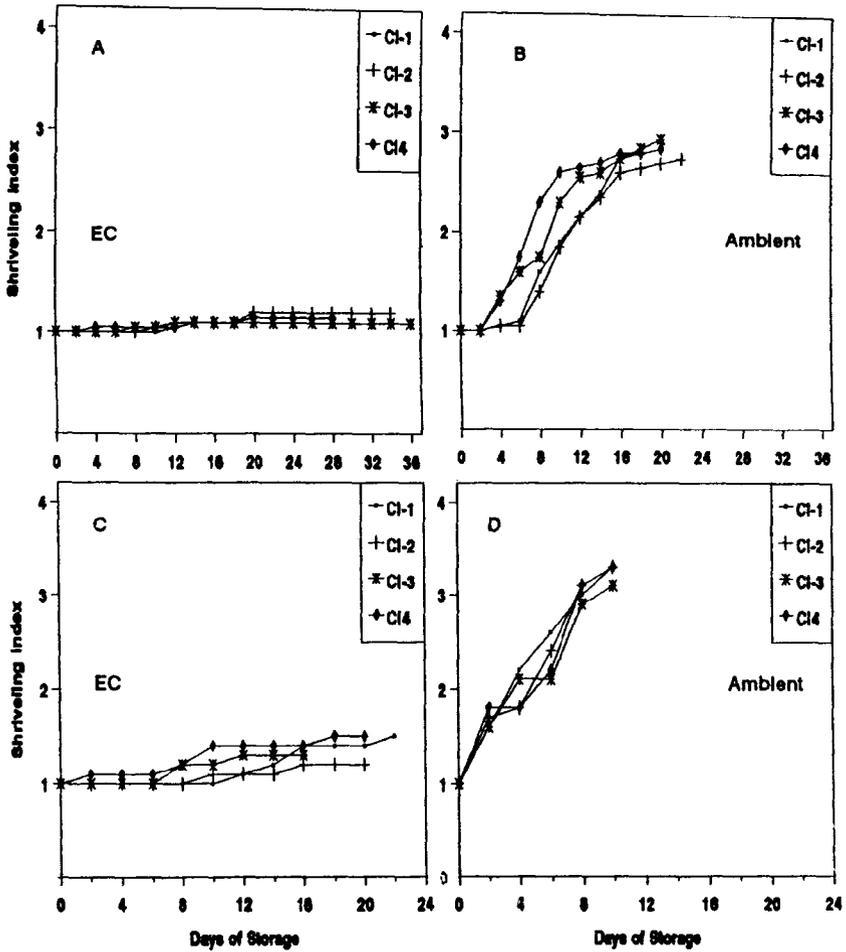


Figure 5. Degree of shriveling of EC- and ambient-stored tomato (A,B) and sweet pepper (C,D) fruits harvested at different stages of ripeness (C1 1-4). (Shriveling index: 1-none; 2-slight; 3-moderate; 4-severe)

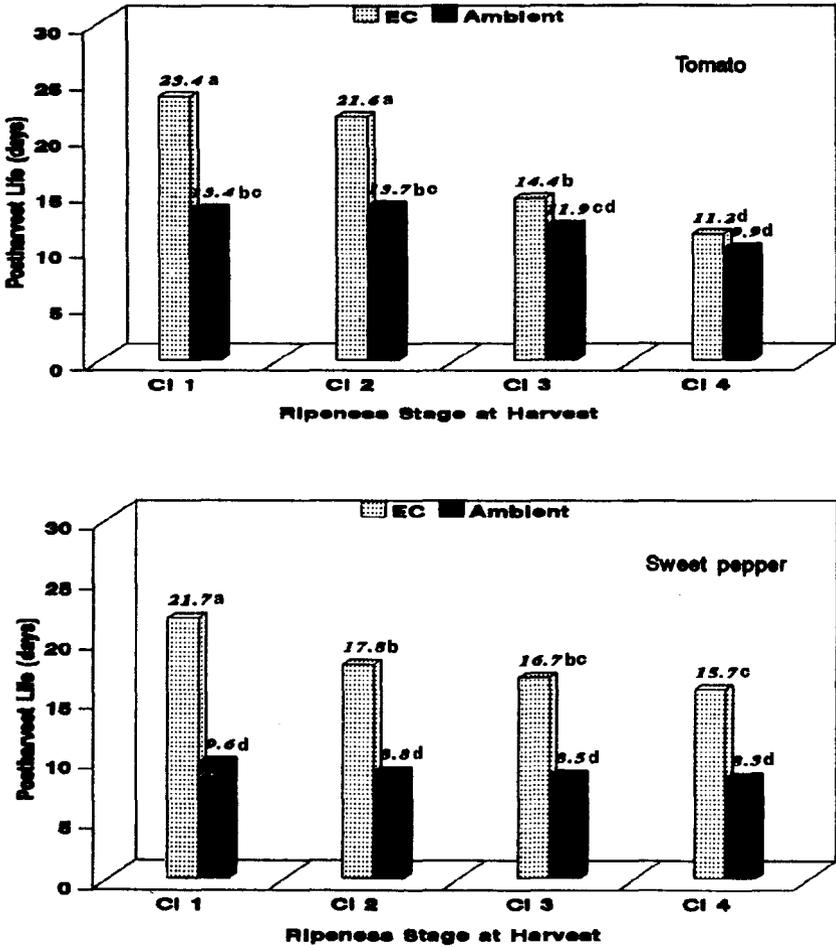


Figure 6. Postharvest life of EC- and ambient-stored tomato and sweet pepper harvested at different stages of ripeness (CI 1-4). Mean separation by DMRT, 5%.

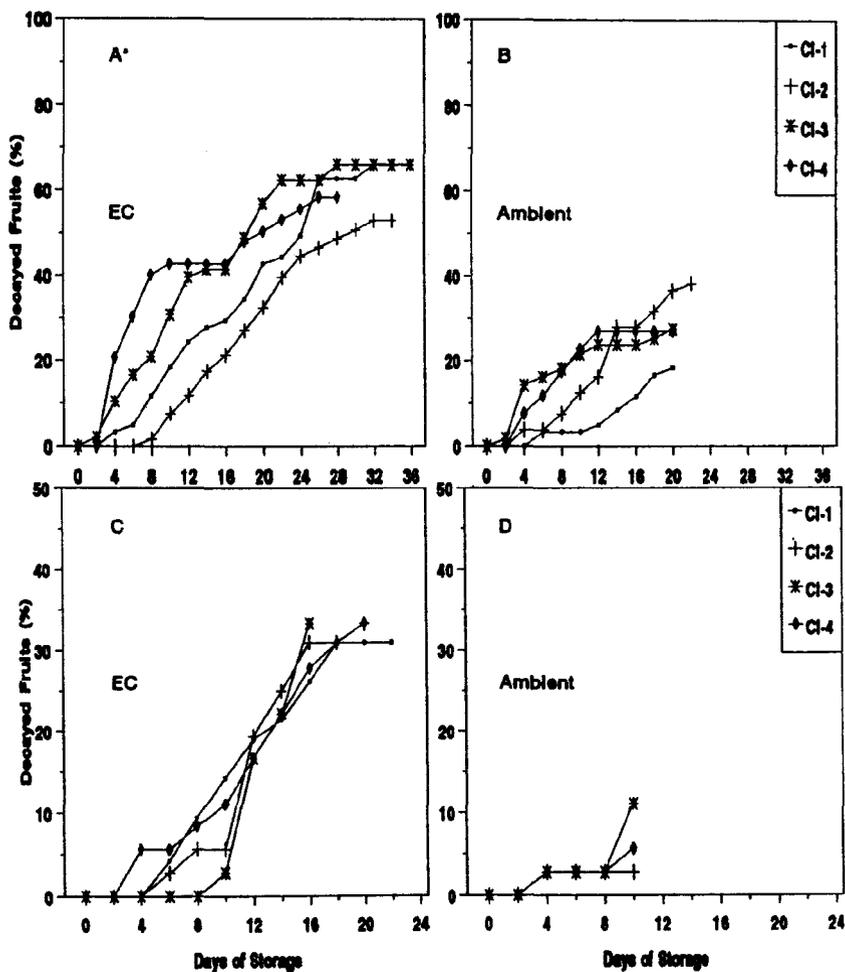


Figure 7. Decayed fruits (percent of total) of tomato (A,B) and sweet pepper (C,D) during EC and ambient storage.



# DEVELOPING SUGARCANE-FRUIT JUICE MIXES

*Rosemarie S. Gumera, Rosita P. Ebron  
Blesilda S. Gregorio, Angelina M. Lojo  
and Marcelino M. Guevarra  
Sugar Regulatory Administration, Quezon City*

## ABSTRACT

Of the nine varieties of sugarcane subjected to taste test, Phil 8013 was most preferred by the taste panel for use as beverage. Six different fruits and three types of preservatives were explored for the development of sugarcane-fruit juice mixes.

The best taste blend of sugarcane-fruit juice mix was 1 part fruit juice, 1.5 parts pure sugarcane juice and 1 part water, by volume, with a total sugar content of 13 to 15 percent. The mixture was pasteurized at 70°C

The best preservative was a combination of ascorbic acid and sorbic acid. Storage at refrigerated temperature (10°C) retained the natural taste and color of the juice better and gave a longer shelf life than at room temperature.

## INTRODUCTION

Natural juices from fruits and sugarcane are excellent refreshing drinks. The development of sugarcane-fruit juice mix offers a more nutritious type of drink. Cane juice is an aqueous solution circulating in the sugarcane plant that carries materials required for growth and metabolism. Studies (2) revealed that sugarcane juice contains minerals such as potassium, magnesium, calcium, aluminum, sodium, iron and manganese, vitamin D, thiamine, riboflavin, pantothenic acid, niacin, biotin, amino acids, stigmasterol, and anti-stiffness factor and myo-inositol a growth factor.

Sugarcane-fruit juice mix is a perishable product and its quality deteriorates on prolonged storage. Changes which can modify the physical and chemical properties of the juice are the result of microbial activity and the presence of gases. To stabilize and to prevent undesirable changes upon storage, preservatives are added and the optimum storage temperature has to be established.

The study determined suitable sugarcane varieties for use as beverage, best taste blend of sugarcane-fruit juice mix and effects of three different preservatives and storage at refrigerated (10°C) and room temperatures on the shelf life of sugarcane-fruit juice mix samples.

## MATERIALS AND METHOD

### Selection of Sugarcane Variety

The nine sugarcane varieties and their code numbers used in the taste test were Phil 6553 (1), Phil 6723 (2), Badila (3), Phil 8093 (4), Alunan (5), Phil 62120 (6), Phil 7228 (7), Phil 8013 (8), and Phil 7544 (9).

Millable stalks were gathered from 12-month old test varieties. The stalks were crushed in a mini-crusher. The extracted juice of each test variety was collected and the brix reading obtained. The juices were diluted with water to get a uniform brix reading of 17. The color of the resulting mixture was noted.

The juices were poured in transparent plastic cups at half the volume. Twenty seven cups were used for each variety.

Twenty seven respondents comprised the taste panel. Each respondent was supplied with a questionnaire.

### Preparation of Sugarcane Juice

Freshly harvested sugarcane stalks of Phil 8013 were cut into pieces of about 0.5 m in length. The rind of the stalks were removed. The juice of the rind-free canes was extracted through a cane crusher and strained through a cheesecloth. The total sugar content, brix and pH were determined. The strained juice was centrifuged at 2000 rpm for 15 minutes.

### Preparation of Fruit Juice

Fresh and fully ripened fruits were selected, weighed and washed. The kalamansi (*Citrus microcarpa Bunge*) were sliced and the juice squeezed out then strained. The mango (*Mangifera indica L.*), soursop or guayabano (*Anona muricata L.*), chico (*Achras zapota L.*), apple (*Pyrus malus L.*) and pineapple (*Ananas comosus M.*) were peeled and cut into pieces. The pulp obtained from these fruits was measured and blended separately. The volume of water added to the pulp upon grinding was 20% for pineapple, mango, soursop and chico and 30% for apple, both based on the total volume of the fruit puree with water.

### Preparation of Sugarcane-Fruit Juice Mix

A series of mixtures of different proportions of sugarcane juice, fruit juices and water was prepared. The mixture was centrifuged at 2000 rpm for 5 minutes and tested for the best taste blend.

Forty samples of sugarcane juice and sugarcane-pineapple juice mix were prepared. The juice samples were treated with three test preservatives, namely, ascorbic acid, sorbic acid and sodium benzoate. The preservatives were added separately to the sugarcane juice and sugarcane-pineapple juice mix at a concen-

tration of 100 mg/liter (100 ppm).

The acidity of the juice mix samples was adjusted to pH 4.0-4.5 by the addition of citric acid. The juice mix samples were packed aseptically in culture tubes with screw caps, pasteurized and exhausted over a water bath at 70 - 80°C for 30 minutes, cooled and sealed tightly. Half of the juice mix was stored at room temperature while the other half was stored at refrigerated temperature 10°C for two weeks. The initial and weekly total plate counts using the AOAC method (1) were determined.

Another set of 112 samples of sugarcane juice and sugarcane-fruit juice mix was prepared for each of the six test fruits. Half of the juice mix samples was treated with ascorbic acid alone while the other half was treated with a combination of ascorbic acid and sorbic acid. The acidity of the juice mix samples was adjusted to pH 4.0-4.5 by adding citric acid. The juice samples were packed aseptically in culture tubes with screw caps, pasteurized and exhausted over a water bath at 70 - 80°C for 30 minutes, cooled and sealed tightly. Half of the bottled juice mix was stored at room temperature while the other half was stored at refrigerated temperature (10°C) for four months. The initial load and monthly plate counts were determined using the AOAC method.

## RESULTS AND DISCUSSION

Data on brix and color of the juices of the nine varieties of sugarcane were shown in Table 1. The yellowish color of Phil 8013 and Phil 7544 which was similar to that of pineapple juice was appealing to majority of respondents with 67% and 56%, respectively. More respondents detected a mild flavor for all the test juices. The mild flavor of Phil 7544 and Phil 8013, however, were acceptable by majority of respondents.

Phil 8013 registered the highest number of respondents (44%) who liked very much its taste. On the overall assessment of respondents as to suitability of the varieties for use as beverage, Phil 8013 and Phil 7544 ranked first and second, respectively.

Badila and Alunan, two popular chewing varieties in the Philippines, were not among those preferred by the respondents. These varieties are good only for chewing because they are juicy and have softer rinds.

It appeared from the results of the taste test that color, flavor and taste of juice were factors that contribute greatly to the preference of respondents.

Results of the taste blend tests of sugarcane and sugarcane-fruit juice mixtures are shown in Table 2. The best taste blend of sugarcane juice alone is 1 part water to 2 parts sugarcane juice, by volume. Sugarcane-kalamansi juice mix has the best taste blend at 1 part kalamansi juice, 6 parts sugarcane juice and 4 parts water, by volume, while the other five sugarcane-fruit juice mixtures have the best taste blend at a ratio of 1 part fruit juice, 1.5 parts sugarcane juice and 1 part water, by volume, with a total sugar content of 13 to 15 percent.

The ratio of the juice constituents varies depending on the type, variety and

maturity of the fruit puree. The pH range of 4.0-4.5 is of medium acid character which required a relatively short time to sterilize the product and inactivate the enzymes and microorganisms present in the juice. At pH values greater than 4.5, severe heat treatment is required to kill spores of microorganisms and such treatment would cause heat damage to the flavor of the juice. By adjusting the pH to below 4.5, less heating is required and flavor changes are minimized.

In the natural state, fruits and sugarcane juices consist of a large number of adventitious microorganisms such as yeasts, molds and bacteria. Total plate count data for pure and fresh juices is shown in Table 3. Pure sugar-cane juice, chico and mango had the highest total plate count, each with 30,000 colonies per ml.

Pasteurization at 70-80°C was found to be sufficient in inactivating the initial load of microorganisms present in fresh fruits provided that the juice is of the medium-acid character.

Based on the results of microbiological analyses of sugarcane juice and sugarcane-pineapple juice mixtures stored for two weeks at room and refrigerated temperatures, the best preservative was sorbic acid. Plate count data is shown in Table 4. Sorbic acid is a 2,4-hexadienic acid and is the only known preservative which is metabolized to carbon dioxide and water (3). Sorbic acid is effective for the inhibition of yeast fermentation, against many common molds and in the suppression of microbial growth. It has the advantage of not impairing the taste of fruit juices (6). The allowable amount is 1 g/liter of 1000 ppm (7).

The juice samples treated with ascorbic acid maintained the characteristic color of sugarcane-fruit juice mix upon storage compared to samples treated with other preservatives without ascorbic acid. Ascorbic acid is effective in preventing the browning of fruits and juices. Normal cane juice, as it circulates in the intact cane, is colorless, however, the tissue breakdown produced in crushing the cane permits the colloidal suspension and solution of pigmented substances not normally present in the juice. The tannins and water-soluble anthocyanins are the major color contributors in cane juice together with the hydrolyzing and color-producing enzyme systems which are active in raw cane juice. Browning of fruits and their juices is caused by the presence of phenolic compounds produced from the oxidation of aromatic compounds present in the plant juice, catalyzed by such enzymes as phenol oxidase and polyphenol oxidase, which then react with ferric ion to produce dark colored complexes. Ascorbic acid acts directly on the functional group of enzymes which cause browning and acts as a reducing agent for the oxidized intermediate of a phenolic compound thus preventing polymerization and subsequent oxidation of a substrate to brown products (4). Ascorbic acid fortified the vitamin C content of the juice.

The shelf life based on total plate count of sugarcane-fruit juice mix samples treated with ascorbic acid alone and those treated a combination of sorbic acid and ascorbic acid and stored at room and refrigerated temperatures is presented in Table 5. Samples stored as refrigerated temperature had longer shelf life compared to those stored at room temperature. Treatment with a combination of sorbic acid and ascorbic acid showed a longer shelf life than those samples treated with ascorbic acid alone.

The retention of the natural taste and color of and control of microbial growth in sugarcane-fruit juice mix samples were generally best at refrigerated temperature and with a treatment of a combination of ascorbic acid and sorbic acid.

Yield data is summarized in Table 6. Based on 1 kilogram of whole fruit and amount of fruit puree extracted, guyabano gave the highest yield, followed by mango, chico, apple, pineapple and calamansi. In terms of total sugarcane- fruit juice mix, mango produced the highest volume, followed by chico, apple, guyabano, calamansi and pineapple. Cost estimate is presented in Table 7. Process flowsheet for sugarcane-fruit juice mix beverage is shown in Table 8.

Newly harvested and unburned clean canes are recommended for use in the manufacture of sugarcane-fruit juice mix beverage. It was observed that juice extracted from cane pith had a lighter and more appealing color than those expressed from intact canes. Automatic removal of cane rind is advisable to get rid of the colorants naturally occurring in the outer layer or cane rind.

Control of the quantity and character of the suspended solids in the juice is one of the most important operations affecting quality. Freshly expressed sugarcane juice should be passed through a centrifuge to remove excess fiber and other materials which may have been carried through the crushing process. The centrifugation of the blended sugarcane and fruit juice should be at a controlled rate to regulate both the amount and size distribution of cellular fragments remaining in the beverage juice. The suspended solids are very important in giving body to the juice and give a discernible effect upon flavor perception by the consumer.

The final diluent for each fruit juice is water and it is essential that it be as nearly pure as commercially feasible. High alkalinity is one of the most undesirable of the water impurities. If the alkalinity is high in water, it will neutralize the acid or destroy the natural taste of the juice and may lessen the effectiveness of the preservative. Fruit juice beverage requires water that meets the standards presented in Table 9.

To improve the shelf life of the juice mix and retention of its natural flavor and fresh aroma, pasteurization of the juice mix by UHT treatment is highly recommended. By UHT treatment, the juice will be initially heated to 80°C during the initial 15 - 20 seconds, then given a heat treatment at 140 - 150°C for 2 - 4 seconds and then cooled rapidly using refrigerated water (5). The use of a Tetra Pak Packaging Machine, a specialized type of packaging equipment presently used by large beverage companies is also recommended in packing the juice mix. The tetra pak package provides a ready to drink beverage with a throw-away container which fits the present trend of packaging juices. A consumer's survey and cost studies should be conducted considering that sugarcane-fruit juice mix is a new product in the domestic market.

**REFERENCES**

1. Association of Official Analytical Chemists. 1984. Centennial Edition. Official Methods of Analysis.
2. Binkley, W. W. & M. L. Wolfrom. 1986. Composition of Cane Juice and Cane Final Molasses. Scientific Report Series No. 15. Sugar Research Foundation, Inc. pp. 4-11.
3. Burmeister, H. 1958. Preservation of raw fresh juice of fruits. *Flussiges Obst*, 25, No. 12, 13-17 (German). Cited in *Fruit and Vegetable Juice Processing Technology*, Donald K. Tressler and Maynard Joslyn, eds. 2nd ed., 1971, pp. 186-233.
4. Charley, H. 1970. *Food Science*. p. 448.
5. Covar, R. 1986. Alternative Products from Sugar. *Sugar Food Products*.
6. Dryden, E. C. and C. H. Hills. 1959. Taste thresholds for sodium benzoate and sodium sorbate in apple cinder. *Food Technol.* 13-86. Cited in *Fruit and Vegetable Juice processing Technology*, Donald K. Tressler and Maynard A. Joslyn, eds. 2nd ed., 1971, pp. 186-233.
7. Saller, W. 1957. Sorbic acid - a new preservative for fruit juices. *Truchsaft-Industries*. 2, 14-19. Cited in *Fruit and Vegetable Juice Processing Technology*, Donald K. Tressler and Maynard Joslyn, eds.

**Table 1. Brix and color of sugarcane juices for the selection of cane variety.**

Sugarcane Variety	Brix	Color
Phil 6553	23.3	Yellowish – Brown
Phil 6723	24.9	Yellowish – Brown
Badila	18.6	Grayish – Green
Phil 8093	24.3	Very light yellowish – Green
Alunan	19.0	Light yellowish – Green
Phil 62120	25.4	Yellowish – Brown
Phil 7228	23.3	Yellowish – Brown
Phil 8013	24.4	Yellow
Phil 7544	23.4	Yellow

**Table 2. Ratio of constituents, pH and % total sugar of sugarcane-fruit juice mix.**

Juice Mix	Purity of Fruit Juice (% Puree, by Volume)	Parts by Volume of Constituents			pH (initial)	%Total Sugar
		FJ*	SJ**	Water		
Sugarcane	--	--	2	1	5.6	14.4
Sugarcane-Pineapple	80	1	1.5	1	4.1	14.8
Sugarcane-Guyabano	80	2	1.5	1	6.3	13.8
Sugarcane-Calamansi	100	1	6	4	2.3	14.3
Sugarcane-Chicos	80	1	1.5	1	6.5	13.4
Sugarcane-Mango	80	1	1.5	1	6.2	13.5
Sugarcane-Apple	70	1	1.5	1	5.6	13.6

\* Diluted Fruit Puree

\*\* Pure Sugarcane Juice, 23°Brix

**Table 3. Microbiological analysis of pure, fresh juices.**

Juice	Total plate count (Colonies/ml)
Sugarcane	30,000
Chicos	30,000
Mango	30,000
Pineapple	7,000
Calamansi	4,000
Apple	2,000
Guyabano (Sour sop)	1,700

**Table 4. Total plate count after two weeks of sugarcane juice and sugarcane-fruit juice mix.**

Juice Samples	Preservative (100 ppm)	Refrigerated Temperature (Colonies / ml)	Room Temperature (Colonies / ml)
Sugarcane	None	10000	TNTC *
Sugarcane	Ascorbic Acid	1200	TNTC
Sugarcane	Sorbic Acid	98	300
Sugarcane	Sodium Benzoate	396	1,000
Sugarcane-Pineapple	None	100	5,700
Sugarcane-Pineapple	Ascorbic Acid	20	100
Sugarcane-Pineapple	Sorbic Acid	0	65
Sugarcane-Pineapple	Sodium Benzoate	38	1,400

\* Too Numerous to count

Table 5. Shelf life of sugarcane-fruit juice mix.

Juice Sample	Preservatives (100 ppm each)	Shelf life (No. of Months)	
		Refrigerated Temperature	Room Temperature
Sugarcane - Water	Ascorbic Acid	2	1
Sugarcane - Water	Ascorbic Acid + Sorbic Acid	3	2
Sugarcane - Pineapple	Ascorbic Acid	3	2
Sugarcane -Pineapple	Ascorbic Acid + Sorbic Acid	4	4
Sugarcane - Apple	Ascorbic Acid	1	--
Sugarcane - Apple	Ascorbic Acid + Sorbic Acid	3	1
Sugarcane - Mango	Ascorbic Acid	3	3
Sugarcane - Mango	Ascorbic Acid + Sorbic Acid	4	3
Sugarcane - Calamansi	Ascorbic Acid	4	3
Sugarcane - Calamansi	Ascorbic Acid + Sorbic Acid	4	4
Sugarcane - Guyabano	Ascorbic Acid	4	3
Sugarcane - Guyabano	Ascorbic Acid + Sorbic Acid	4	4
Sugarcane - Chicos	Ascorbic Acid	3	3
Sugarcane - Chicos	Ascorbic Acid + Sorbic Acid	4	1

Table 6. Yield data of sugarcane-fruit juice mix beverage based on 1 kilogram of fruit.

Fruit	Volume of Puree (ml)	Diluted Puree (ml)	Pure Sugarcane Juice (ml)	Water (ml)	Total Juice Mix (ml)
Pineapple	500	625	938	625	2185
Guyabano	1000	1250	938	625	2813
Mango	900	1125	1688	1125	3938
Chicos	800	1000	1500	1000	3500
Apple	800	1000	1500	1000	3500
Calamansi	240	240	1440	960	2640

Table 7. Cost estimate of raw materials for the manufacture of sugarcane-fruit juice mix beverage.

Type of Fruit	Cost/ kg Fruit	Cost of SJ* per kg fruit	Total Volume of Juice Mix (ml)	No. of Packs (200 ml /pack)	Cost of Packing Material **	Total Cost	Cost / Pack
Pineapple	P 10.00	P 0.78	2188	11	P 22.00	P 32.78	P 2.98
Guyabano	10.00	0.78	2813	14	28.00	38.78	2.77
Mango	20.00	1.40	3938	20	40.00	61.40	3.07
Chicos	20.00	1.25	3500	18	36.00	57.25	3.16
Apple	25.00	1.25	3500	18	36.00	62.25	3.46
Calamansi	10.00	1.20	2640	13	26.00	37.20	2.86
Sugarcane (1 ton)***	--	500	600,000	3,000	6,000	6,500	2.17

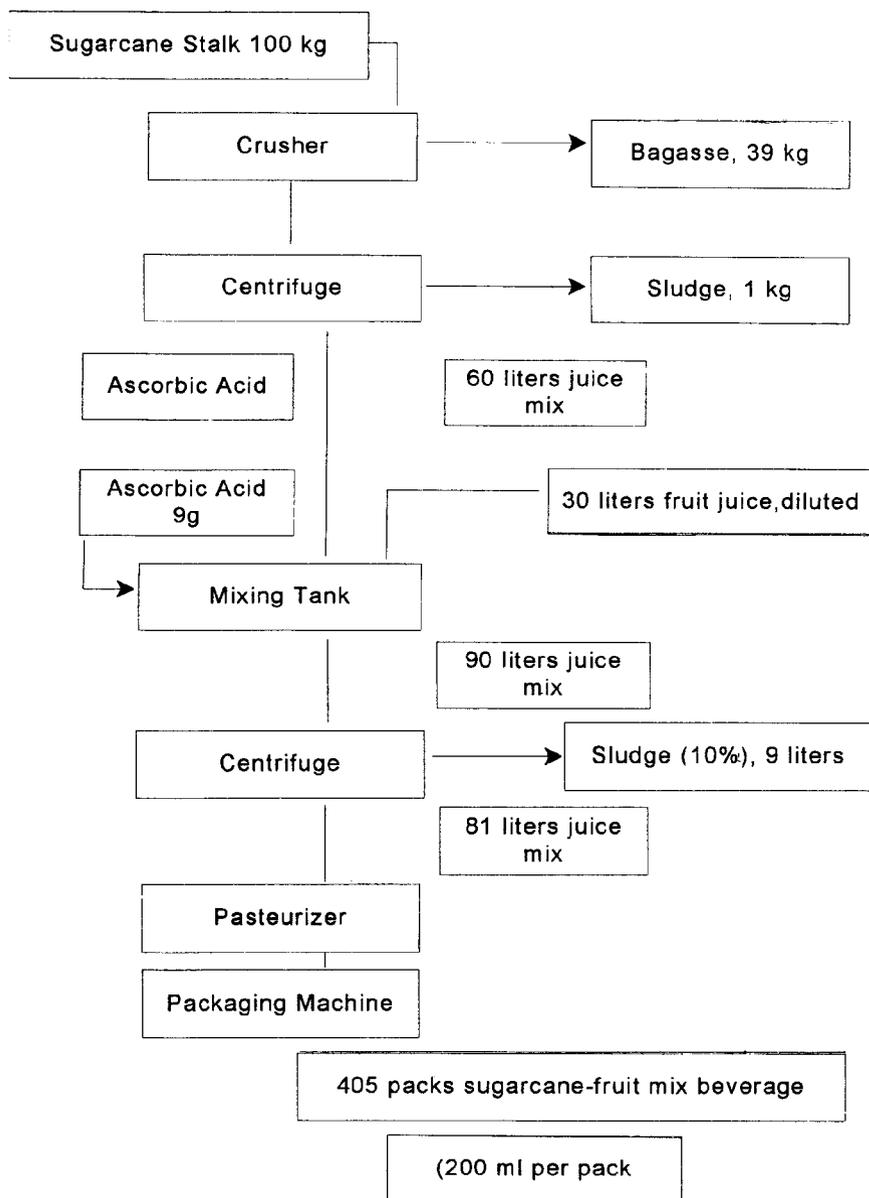
\* Pure Sugarcane Juice

\*\* Cost of packing material for the juice - P 2.00 / pc

\*\*\* Approximate price of sugarcane is P 500.00 / ton

**Table 8. Process flowsheet for the production of sugarcane-fruit juice.****Mix Beverage**

BASIS: 100 kg Sugarcane Stalk: 60% extraction



**Table 9. Laboratory standards for water to be used in preparing fruit juice beverages.**

Property/Component	Maximum Allowable Amount
Alkalinity	50 ppm
Total Solids	500 ppm
Iron	0.1 ppm
Manganese	0.1 ppm
Turbidity	5 ppm
Color	Colorless
Residual Chlorine	None
Odor	None
Taste	No Off Taste
Organic Matter	No Objectionable Content

**Second Plenary Session**  
**FOOD IRRADIATION**



# FOOD IRRADIATION: INTERNATIONAL TRADE OPPORTUNITIES

*George G. Giddings and Paisan Loaharanu  
Food Preservation Section  
Joint FAO/IAEA Division of Nuclear Techniques  
in Food Agriculture  
International Atomic Energy Agency Vienna, Austria*

## ABSTRACT

After decades of research and development, safety/wholesomeness evaluation accumulating national approvals, public debate, and slow, gradual industrialization, food irradiation is now moving to center-stage to join more established food technologies in helping ensure global food safety and security. Among the foods that stand to benefit from this technology, and already are to some extent, are the following: (a) foods of animal origin (meats, poultry and fishery products) that can be rendered far less likely to cause parasite and pathogen infection, and be lost to spoilage; (b) fresh and dried fruit and produce including nuts, etc., that can be rendered free of insect pest concerns, thus fostering international trade, as well as becoming longer-lasting in the case of perishable products (fresh berries, tubers, climacteric fruits, mushrooms, etc.); (c) grains and other relatively dry and stable stored products that require or would benefit from an insect pest disinfestation treatment; and last but-not-least, spices and other dry ingredients and the like that are common carriers of insect pest and high levels of microorganism contamination including pathogens. This most versatile of all food processing/treatment technologies can accomplish all these benefits, and more, in contributing to food safety and security, and to global trade in food commodities and products. As the title indicates, this paper focuses primarily on global trade, including the implications of such factors as increasing national insistence on imported foods meeting ever stricter quality and safety standards; international trade agreements and related (e.g., GATT, NAFTA, EU agriculture - food policies), and the gradual phasing-out of post-harvest chemical treatment of foods and ingredients. Such factors auger well for the future of global trade in irradiated foods and their raw materials and ingredients.

## INTRODUCTION

Two years from now, 1996 will mark the passage of one-hundred years since the appearance of the first known publication on the effect of ionizing radiation on bacteria, in a German weekly medical publication, that is often credited with "laying-the-cornerstone" of what was to become the radiation preservation of perishable food through its lethal effect on microorganisms. Early research, and even a couple early patents on radiation preservation of food date back to shortly after the last turn-of-the-Century. The first primitive animal feeding studies to

test the toxicological safety of irradiated foods were conducted and published in the 1920's, making these among the earliest uses of the "rodent bioassay" method of safety testing. Through the overwhelming bulk of food irradiation R & D has been done during the past 30-40 years, it is a rather remarkable irony that food irradiation was conceptualized, studies and published on, and even (prematurely) patented long before food and ingredient chemical fumigation, microwaving, aseptic and controlled/modified packaging, etc., came on the scene; yet to most people food irradiation is a new and unfamiliar technology whereas these newer ones are relatively commonplace and familiar.

This is because until recently there has been a dearth of irradiated foods in the global marketplace, and while on the increase of late it is still but a trifle. So, there has been very little product, and in most countries still none whatsoever to capture public attention. What has gotten newsmen and the public's attention has been the largely political (as opposed to scientific) controversy that has been raging internationally for the last ten years-or-more, fed and fanned by anti-nuclear and other special interest activists endeavoring to advance their misplaced political agendas by attacking food irradiation to the exclusion of nonfood applications. This most bizarre and intense food-related controversy is not over yet, but the tide of media and public perception has markedly improved since the "dark" days of the mid-to-late 1980's when outrageous activist propaganda was shaping media, public and political opinion. But as fact has overcome fiction in the arena of public debate, and as the appearance of increasing amounts of "high-profile" irradiated foods in the marketplace has focused media and public attention on its public health and food availability and quality benefits, media coverage and public perception has become much more accurate and positive, and the end-is-in-sight' for a largely contrived controversy that has delayed regulatory approvals and industrial utilization of food irradiation, but was doomed to failure to stop progress.

Accepting this, the attention of advocates of the prudent, rational industrial use of this proven beneficial technology has turned from that distraction to seeing to it that food irradiation takes its hard-earned place among the more traditional and established food processes/treatments. In this context, in addition to furthering public awareness through regional public information seminars for opinion-shapers, helping put in place the legal-regulatory and standards, etc. framework for, and otherwise facilitating global trade in irradiated foods and their raw materials and ingredients has moved to the forefront of the Joint FAO/IAEA Division's Food Preservation Section and activities. The following is a brief, somewhat speculative summary of what are perceived at this point in time to be the real international trade opportunities, now and in the foreseeable future, for irradiated foods and their raw materials and ingredients on a commodity-by-commodity or food class basis, and some of the reasons why it appears to be the case.

## **GLOBAL TRADE OPPORTUNITIES**

**Animal Products:** Food products of animal origin include edible meat, poultry and fishery products as distinct from plant products (fruit, vegetable, grains,

nuts, etc.). Fresh, frozen dried and otherwise processed (e.g., ham, sausages) red meat products have been traded within and among regions for a very long time, but the outlook for international trade in so-called red meats (beef, pork, lamb/mutton, horse, etc.) that have been irradiated is less clear at this time than it is for poultry, and especially fishery products that have already seen some global trade (e.g., radiation pasteurized frozen Asian shrimp and frog legs.) At the present there are relatively few approvals of red meat product irradiation compared with irradiated poultry approvals now in place worldwide. This may-or-may-not reflect the relative interest in the three groups as irradiation candidates, especially for trade.

The two distinct but interrelated main reasons for irradiating meat are (1) to reduce the likelihood of parasite and/or microbial pathogen infection and intoxication, and (2) to extend the edible-marketable life of perishable meat products (fresh or semipreserved), normally in combination with refrigeration, by reducing levels of common nonpathogenic spoilage microorganisms. Though quite different objectives, they are interrelated because both are microbial reduction applications that occur simultaneously no matter which is the primary or only real objective. Also, both will result in the elimination of any parasite threat, though this can be achieved independently at a much lower radiation dose than for 1 and 2. Though these treatment effects can certainly support trade in meats, it seems at this time that the dominant interest, where it exists (e.g., in the USA where a red meat irradiation approval petition is being acted upon by the USDA with strong industry support) is for fresh and frozen raw meat pasteurization against microbial pathogens for the domestic market. A couple specific exceptions could be the radiation pasteurization of exported ground beef to eliminate pathogen contamination and thereby meet importer specifications, and irradiation of raw meat normally shipped long distances as frozen (e.g., Australian/N.Z., lamb) to permit less expensive refrigerated shipment of potentially higher value nonfrozen meat (i.e., a double economic incentive if one can ship more cheaply and also realize a higher price.

Poultry meat, either as intact dressed carcasses, cut-up parts and/or mechanically deboned/separated minced poultry meat appears at this point in time to be more likely to be traded as irradiated products than the red meats in the foreseeable future. Generally speaking, raw poultry has more-or-less the same microbial pathogen problems as the red meats, differing only in details, while being comparatively parasite-free. The two classes also share much the same spoilage microflora and are about equally perishable in the raw chilled/refrigerated state. So, raw, fresh red meat and poultry both benefit in the same way, and to about the same extent from a radiation pasteurization treatment. Some mechanically deboned, minced poultry meat, radiation pasteurized as frozen slabs to eliminate *Salmonellae* and other microbial pathogens that might be present, has been exported from France for several years. Interest has been shown in doing the same in the U.S. since raw fresh and frozen poultry irradiation was fully approved in late 1992, but no mainstream poultry industry adoption has been taken as yet. Small quantities of intact dressed chicken carcasses and parts supplied by an irra-

diation firm have since been successfully sold in the U.S. domestic market, however.

Elements of the Brazilian poultry, a major exporter of frozen poultry to the Middle East and elsewhere, have also shown interest in radiation for both the domestic and export markets, but here again no concrete action has been taken beyond doing and supporting studies. In principle, radiation pasteurized raw poultry in chilled/refrigerated form could displace frozen surface shipments to the middle East, for example, with enough remaining edible-salable life to be satisfactorily marketed and consumed as the higher valued of the two forms. As in the above lamb example, the industry could realize the double benefit of saving on shipping costs, refrigerated being cheaper than frozen, while gaining higher wholesale/retail prices. Other major poultry exporters such as Western Europe and the United States could of course do likewise, so time-will-tell how fast and to what extent global trade in irradiated poultry takes place. It does appear certain, however, that it will take place to a quite significant extent in the not-too-distant future, and probably to a greater extent than for the red meats although that, of course, remains to be definitively determined.

Of the three major animal products classes, irradiated edible fishery products are probably the most likely to experience relatively rapid, widespread growth in international trade in the years ahead. Among the reasons for this expectation is the fact that, much more so than meat and poultry, fishery products tend to move in international trade from the less developed to the more developed countries. Other reasons are that fishery products are generally more perishables than, and at least as likely to harbor infectious parasites and microbial pathogens, if not more so, than meat and poultry. Already for a number of years, radiation pasteurized frozen Asian seafood has been entering European, North American and perhaps some other market, often illegally for lack of regulatory clearances. The reason for this can truly be expressed as a case of clear and present need to disinfect such products of harmful microorganisms meeting the only available method of doing so without changing the nature and state of the products, but before regulatory approvals have caught up with the need and irradiation's availability. That is, one can say that this situation is more a case of a lag period between the inevitable solution to a need and its approval than a serious violation of laws and regulations. Put yet another way, it can be regarded as a case of the temporary absence of needed approvals rather than the presence of, and violation of explicit prohibitions.

Probably the best example of a combined public health and economic benefit from the irradiation of a major edible fishery product that is traded internationally is that of marine shrimp. Significant quantities of frozen shrimp coming out of Asia, from India to Indonesia, have been and continue to be irradiated, mainly in Europe, to rid these products of microbial pathogen contamination that has been connected with serious food poisoning outbreaks. As good manufacturing practices (GMP) continue to improve at the sources this may be expected to become less-and-less needed, but may not become completely unnecessary. In any case, the same irradiation dose that is applied to frozen shrimp, or somewhat

less, can extend the edible-marketable life of chilled/refrigerated shrimp through spoilage microorganism reduction such that it could allow non-frozen surface shipment to export markets, just as for the above meat and poultry cases. And because microorganisms in general including pathogens tend to be more radiation-sensitive in the thawed than in the hard frozen state, one can expect at least as great a degree of pathogen kill, and perhaps more so, at a somewhat lower dose applied to chilled than the typical dose applied to frozen. Besides eliminating the costs of freezing and frozen storage, the lower dose means a lower irradiation cost.

Even if the primary purpose of irradiation were chilled/refrigerated market life extension, it should at the same time be at least as effective against pathogens as is irradiation of frozen shrimp, thus the combined benefit. Block-frozen shrimp is largely traded on price as a 'spot' commodity, which tends to depress revenues to the producer, mainly developing countries. In contrast, the tiny fraction that is exported as fresh chilled (e.g., by air from Ecuador to upscale New York City restaurants) is treated as a high price value-added product for not having been frozen. If radiation pasteurized, the delay of onset of spoilage could allow refrigerated shipment by seagoing vessel instead of by air, thus saving shipping costs. Similarly, further processors of raw frozen shrimp into breaded-cooked branded products, for example, almost invariably buy the shrimp on price as a commodity, thaw and process it, and refreeze the finished product. This double freezing has a negative effect on eating quality, especially texture but also flavor, and it is well known that if the original shrimp were non-frozen, as through radiation pasteurization, the frozen finished product would be superior, and could be marketed as such for a higher price. This plus the savings from avoiding freezing and frozen shipping/storage of the original shrimp should far more than offset the cost of irradiation. This is but one example of the potential of irradiating an already traded seafood. There are, of course others in the fresh, frozen and dried categories.

**Plant Products:** Again, this class of course includes fruits, vegetables, grains, legumes, tree, bush and ground nuts, and spices and herbals-and-the-like, just to name some. As in the case of fishery products, global trade is to a large extent from developing to the more economically/industrially developed countries. Many edible plant products share one thing in common: namely that they require or at least benefit from a post-harvest insect pest control treatment. Major examples are methyl bromide fumigation of considerable quantities of fresh and dried fruits, vegetables and nuts for export to countries with insect pest plant protection/quarantine restrictions; phosphine fumigation of grains and legumes for protection against insect pests during long-term storage or for export; and, ethylene and propylene oxide fumigation of a host of spices and other dry food ingredients for both insect pest and microorganism control. What with the continuing and even accelerating trend of drastically-restricting-to-completely-curtailling such uses of toxic chemical post-harvest fumigants for environmental (e.g., air quality), worker safety (inhalation and skin contact exposure) and residues in/on the product restricting-to-completely-curtailling such uses of toxic chemical

post-harvest fumigants for environmental (e.g. air quality), worker safety (inhalation and skin contact exposure) and residues in/on the product reasons, they are truly a 'vanishing breed' with one after another being banned or so severely restricted that continued use is impractical.

While in most cases there are other treatment alternatives, generally speaking irradiation is being increasingly regarded as the technically all-around best of them in terms of its unique effectiveness against insect pests and microorganism, its versatility in the very wide range of its proven applications, and, its relative harmlessness towards the products in question. Nevertheless, a general reluctance among agroindustries to adopt technologies that are perceived as unfamiliar and intimidating and, of course, controversial and expensive, coupled with slowness on the part of regulatory authorities to put in place regulations permitting irradiation to be applied as a fumigation alternative in all but the spice/ingredient case has seriously slowed or delayed its adoption for all those other uses. However, the regulatory obstacle is gradually being lifted at the same time that most other treatment alternatives are being found wanting in practice for effectiveness, product damage and/or cost reasons. So, while it still remains to be seen how quickly and to what extent irradiation will become the fumigation alternative of choice, the outlook is steadily improving as the regulatory and the unfamiliarity and controversy-related impediments to adoption dissipate.

In addition to insect and microorganism control, there are many actual and potential applications of irradiation to plant products for the delay of physiological (e.g. potato/onion germination-sprouting, climacteric fruit ripening-senescence) and microbiological (e.g., molding of berries and other fruit) spoilage in order to extend edible-marketable life. In the case of most climacteric fruit (mango, papaya, etc.) for example, a single irradiation treatment can fulfill any quarantine treatment requirement and simultaneously delay ripening/senescence thus extending edible-marketable life. Only irradiation can offer that combined beneficial effect, and in fact competing heat quarantine treatments typically accelerate physiological spoilage and shorten life. There is already considerable trade in tropical and semitropical fruit and produce needing a quarantine treatment and in need of longer market life, mainly from developing to developed countries. Further, there is considerable potential for expansion in already established markets such as North America and Western Europe, and in newer markets such as Eastern Europe and the CIS. Irradiation appears now about to begin contributing to keeping existing trade on-going and realizing this growth potential.

Trade in irradiated spices and other dry or dehydrated food ingredients has been growing slowly-but-surely over the last ten years-or-so. This growth has started to accelerate since EU member and other European countries recently banned ethylene oxide fumigation of spices. Even countries that do not yet allow production or import of irradiated foods per-se do permit the import of irradiated spices by regulation (e.g., Finland) or de-facto. Canada and the U.S. permit the use of irradiated spices-and-related; but since they continue to permit ethylene and propylene oxide fumigation the trade essentially only uses irradiation in

cases in which fumigation is unable to bring the product within specifications (e.g., heavy mold contamination) or for potentially toxic residue reasons (e.g., salt-containing spice blends). Now both countries, and perhaps other appear to be about to follow the EU and completely ban fumigation, with North America accounting for massive spice imports. Here, again, irradiation will soon likely contribute to both maintaining existing trade, and, facilitating its expansion since it is more effective and more widely applicable than fumigation.

In conclusion, the next Century can be expected to be the epoch during which food irradiation, which has taken nearly one-hundred years to reach where it is today from conception, will realize its full potential in contributing to global food safety, security, and, trade as trade agreements like the GATT and others now (e.g., NAFTA) and to come likewise reach their full potential in minimizing trade barriers.

**Table 1. Benefits and limitations of irradiation of horticultural crops.**

Commodity	Desired effect	Dose (Gy)	Undesirable effects	Alternatives
<i>Fruits</i>				
Banana	delayed ripening	150-350	skin damage	modified atmospheres low temperatures
Mango	disinfestation	100-250	pale color	vapor heat treatment
Papaya	disinfestation delayed ripening	150-375	uneven pale color	vapor heat treatment modified atmospheres
Strawberry	disease control	375-2000		modified atmospheres
<i>Vegetables</i>				
Onion	delayed sprouting/ rooting	30-100	bud browning	maleic hydrazide
Garlic	delayed sprouting/ rooting, insect control	93	possibly some loss of flavor at > 100 Gy	
Ginger	delayed sprouting			
Potato	delayed sprouting	60/150		
Asparagus	growth inhibition	50-100	tissue breakdown	appropriate packaging temperature/humidity control
Mushrooms	delayed senescence (cap opening color)	-1000	off-flavour	temperature/PH control modified atmospheres
Cucurbits	disinfestation	150-300	abnormal ripening	vapor heat treatment

*Modified from: Council for Agricultural Science and Technology (USA). 1989*

**Session 3**  
**Food Preservation**  
**Technologies**



# PROSPECTS OF FOOD IRRADIATION IN THE HANDLING OF HORTICULTURAL PERISHABLES

*Ma. Concepcion C. Lizada, Ph.D.  
Postharvest and Research Center  
University of the Philippines Los Baños*

## ABSTRACT

The considerable growth in demand for fresh fruits and vegetables in recent years can be attributed to an increased awareness of their contribution to health and general well-being. In horticultural marketing, fresh produce (i.e. products marketed in viable state) presents the greatest challenge in terms of successfully conveying the product from the farm to centers of consumption. Several technologies have been developed to reduce postharvest losses in horticultural produce. These include refrigeration, controlled or modified atmospheres, as well as chemical and other physical treatments. The benefits gained from the application of these technologies, which include reduction of losses due to accelerated deterioration and the extension of post-harvest life, can also be derived from appropriate irradiation treatments of specific horticultural produce.

This paper will focus largely on developments in the Philippines in relation to the potential use of irradiation in enhancing the output derived from the marketing of horticultural produce. However, information from other countries will also be mentioned. In addition it will highlight some of the issues raised in conjunction with the use of irradiation on horticultural produce.

## The 'Manila Super' Mango

The 'Manila Super' mango, also called 'Carabao' in most local markets, is the only cultivar exported by the Philippines. A number of countries require quarantine treatments against the Oriental fruit fly, *Bactrocera dorsalis* Hendel. Studies on mango irradiation, conducted from 1964 to 1990, have concentrated on the potential of gamma irradiation in two areas: disinfestation and postharvest life extension. The radiation doses studied ranged from 160 to 800 Gy (DOST, 1990). Earlier studies reported varying results with respect to fruit response as reflected by quality and ripening behavior. Observations in these studies include the following:

1. delayed ripening in some but not all irradiated fruits
2. discoloration, particularly after refrigerated storage
3. inconsistent disease control

The reported observations might be attributed to such factors as fruit maturity as well as the mode of application of other treatments such as the hot water treatment for disease control and refrigerated storage.

Our work on the irradiation of mango did not start until 1989, a time when we had developed a simple, but more reliable method for determining maturity, established the parameters to ensure efficacy of the hot water treatment (HWT) without incurring heat injury, characterized the response of the mango to low temperature regimes and standardized our methods for monitoring various aspects of ripening.

Working on the findings of Manoto et al (1990) that a minimum dose of 100 Gy is required to disinfest the 'Carabao' mango of the fruit fly, *Bactrocera dorsalis*, we examined the effect of irradiation on the postharvest behavior of the mango. In a series of studies (Lizada et al, 1991) we made the following observations:

1. Irradiation of mango at a dose of 100 to 350 Gy was not reliable treatment for disease control. Most of our studies actually showed no significant control of postharvest disease with the above doses.
2. Based on pulp softening, respiration and internal ethylene, irradiation effected no consistent or significant inhibition of ripening. An apparent inhibition was associated with a mere retardation of the loss of green peel color.
3. At a dose of 350 Gy, persistent patches of green remained in the peel even at the table ripe stage.
4. Unlike vapor heat treatment (VHT), irradiation induced no internal breakdown in the mango; however, pulp discoloration was observed at a dose of 350 Gy.
5. Some sensory attributes, such as peel and pulp color and texture were slightly affected; however, all irradiated fruits were acceptable.
6. There was no significant decline in the vitamin C content of the ripe fruit.

Our recommendations for the use of irradiation include the following:

1. Cull out immature fruits as these are more susceptible to chilling injury, particularly when subjected to the hot water treatment prior to cold storage.
2. Subject the fruit to hot water treatment prior to irradiation to ensure good and reliable disease control and better peel and pulp color.

3. As mangoes are susceptible to chilling injury at temperatures  $< 12.5$  oC, and irradiation might aggravate the injury symptoms, cold store irradiated fruits only at 13-15 oC, making allowances for temperature fluctuations.

Based on the efficacy of 100 Gy in disinfesting the mango of the fruit-fly, and the acceptability of fruits irradiated at a dose of 250 Gy (which is 2.5 times the minimum dose), gamma irradiation should be an acceptable quarantine treatment. Furthermore, it has been demonstrated that no mutagenic radiolytic product is generated and persists in mangoes irradiated at 500-750 Gy (Banzon et al, 1988)

Currently the Philippines has 4 commercial VHT chambers operating for the quarantine treatment of fruits exported to Japan. Although the United States has expressed interest in importing 'Manila Super' mango, the lifting of the ban on Philippine mangoes will depend on the response of the US Department of Agriculture to the survey done on the island of Guimaras. It is interesting to note that, in contrast, the proposed rules for the United States Federal Register on the Importation of Fruits and Vegetables for 1992 included the proposal to allow Taiwanese mangoes which have been subjected to VHT. In March of this year (1994), the proposed rules covered the use of VHT on Mexican mangoes. It appears that unless an importing country specifically requires irradiation as a quarantine treatment, the prospects of mango irradiation are not too bright.

### **'Solo' Papaya**

Based on the information that the minimum dose required for the effective disinfestation of the 'Solo' papaya of the Oriental fruit fly is 150 Gy (Manoto, personal communication), we examined the response of these fruits to gamma irradiation at 150 Gy and 375 Gy.

As with the mango, gamma irradiation at either dose showed no significant effect on disease development. Although the delay in peel color development was not as pronounced as in the mango, green patches that persisted in the peel through ripening were also observed. Softening was significantly inhibited in fruits irradiated at 375 Gy at the color break stage, which became table ripe 13.3 days after treatment. In contrast, control fruits softened to the table ripe stage 10.6 days after treatment. Despite some reduction in peel color intensity, neither visual quality nor acceptability of the pulp was affected.

The target export market for the 'Solo' papaya is Japan, which requires that fruits be subjected to a VHT protocol prior to packaging. This country has not considered irradiation as a quarantine treatment for imported fruits.

## OTHER HORTICULTURAL PRODUCE

### Onion

A dose of 30-100 Gy has been found to inhibit sprouting, mold growth and decay during the cold storage of 'Red Creole' onions (Gonzalez et al, 1969; Singson et al, 1978), such that the proportion of good bulbs increased by 29-45% more. Similarly, for the 'Yellow Granex' the proportion of good bulbs increased by 32%.

Irradiation of Japanese cultivars indicated injury manifested as browning of small inner buds (Kawashima et al, 1990). This disorder as well as measures to ameliorate the symptoms need to be studied in Philippine-grown cultivars.

Although it has been concluded that increased incomes from onion trade in the Philippines can be realized with the use of irradiation (Singson, 1990), other technologies which give comparable results are currently available. Maleic hydrazide combined with curing, for example, has been employed to inhibit sprouting during long-term storage of onions. Economic feasibility studies for onion irradiation should, therefore, include a parallel evaluation of a combined treatment of maleic hydrazide spray, harvesting at the right stage of maturity, proper curing and sorting. Some onion exporters actually apply this combined treatment and have expressed satisfaction with its effect on the marketability of the bulbs.

### Garlic

Garlic cannot be subjected to cold storage as it has a greater tendency to sprout under cold storage than onions. Normally they are subjected to non-refrigerated storage in areas where they are protected from extremely high temperatures with adequate ventilation. However, under these conditions, insect infestation, desiccation of cloves and decay can be a problem, and a cumulative weight loss range of 35-49% is typical (Jarcia and Revilla, 1990).

As yet, no treatment has been successfully applied under commercial conditions to reduce these losses. Only irradiation at a dose of 93 Gy has been reported to effect a reduction of losses in stored garlic (Singson, 1990). This is < 100 Gy, which has been demonstrated to have no significant effect on diallyl disulfide, a key flavor component of garlic (Kwon and Yoon, 1985). Garlic is one crop where the use of irradiation might be attractive in the Philippines.

## ACCEPTABILITY OF IRRADIATION AS A TREATMENT FOR HORTICULTURAL PRODUCE

Thus far, progress in efforts directed towards the commercialization of irradiation in the Philippines has been relatively slow. As with other postharvest technologies, adoption depends, to a large extent, on the need for the particular technology. In evaluating such technologies, the technical feasibility, economic

viability and acceptability among the end-users. Although technical feasibility in relation to disinfestation (for mango and papaya) and sprout inhibition (for onion and garlic) has been demonstrated, the economics of commercializing the treatments in the Philippines have to be studied more thoroughly. It is only after the economic viability of irradiation has been established will investors be interested. As with other technologies, once investors are convinced, they will take on the task of applying and promoting the acceptance of the technology.

Although studies have also demonstrated the safety of irradiation at the recommended doses, which particularly for horticultural produce are relatively low, acceptability still remains a problem. In an earlier marketing study on irradiated onion and garlic (Lustre et al, 1985), however, it was pointed out that many consumers did not react to the label "irradiated" probably due to ignorance. The need for consumer education was stressed. Perhaps we can learn from the experiences of an Illinois supermarket that sells irradiated fruit and chicken for which consumer demand has been positive (Food Business, 1993). The owner attributes this response to the fact that consumers in the area "tend to be well-informed", and "understand irradiation and what it can do".

Timely implementation of a program in consumer education is necessary in anticipating the effects of those who oppose the use of irradiation on the basis of concerns frequently raised (Fazal, 1985; DOST, 1990):

1. safety in relation to the question regarding induced radiation, long term toxicological and mutagenic effects;
2. possibility of creating mutant and resistant bacteria;
3. cosmetic effects on poor quality products;
4. effects on nutrients;
5. enforcement of standards to ensure worker safety;
6. impact on small farmers and small-scale industries; and
7. total cost, including "foreign exchange, capitalization, opportunity, social and environmental costs".

It appears that an objective, impartial approach, providing both pros and cons of food irradiation should be more helpful than attempts to paint only the benefits of irradiation. The latter will only serve to heighten suspicions regarding food irradiation.

In a meeting in Jakarta in 1991, the key role that the irradiation of spices might play was discussed. This view was reiterated in the last RCM on Asian Regional Cooperative Project on Food Irradiation in Taejon, Korea last year. Among food products that can be irradiated, spices are the most likely to be more

easily accepted because of the following reasons:

1. They are usually used in small amounts;
2. Alternatives include such fumigants as ethylene oxide; and
3. Mycotoxins might be present in untreated spices.

Although spices are not classified as produce, they are nevertheless horticultural products. It appears that the irradiation of dried spices, along with garlic and onion, might serve as appropriate products for introducing and gaining acceptance for irradiation of horticultural produce.

# GAMMA IRRADIATION OF SELECTED SPICES

*Zenaida M. De Guzman, Jean M. Casyao,  
Mitos M. Tolentino and  
Luzviminda M. Ignacio*

## ABSTRACT

This study was done to evaluate the efficacy of gamma radiation in the reduction of microorganisms and to determine the keeping quality of commercial spices, namely, black and white pepper, onion and garlic powder, nutmeg and coriander. The spices were supplied by the food industries and were irradiated at varying doses of 0, 3, 6 & 9 kGy. Microbiological, physicochemical and sensory evaluations were done. The exposure of spices to a dose of 6 kGy reduced the microbial load to as much as 3 log cycles for total plate counts and 2-3 log cycles for total mold and yeast counts. The moisture content of irradiated and non-irradiated spices ranged from 6.0 to 10.0 percent, respectively. Irradiation up to a dose of 9.0 kGy did not cause detectable change in color and flavor of the samples. Microbial reduction observed in irradiated black and white pepper was maintained during a storage period of six months.

## INTRODUCTION

Spices are used as raw materials and ingredient in semi-processed and processed meats, fish and bakery products in most food industries. Although spices are employed as minor ingredients (of the order of 0.1-2%) in food products, they nevertheless are significant sources of microorganisms in many processes foods. Microorganisms in spices are introduced during harvest and food processing and occasionally produces toxic effects (1).

The spice production in the Philippines for the last five years (1989-1993) averaged 26,120 MT valued at P1,314 Million while the average export is 160,688 kilos (2). A greater volume of the spices produced is consumed locally. The local consumption of the spices is supplemented by importation (Table 1) from 23 countries, principally from U.S.A., Singapore, Hong Kong and Switzerland (3).

Samples of locally produced spices were heavily contaminated with microorganisms and some of the samples were infested with insects. The common method of decontamination is by treatment with ethylene oxide (ETO). However, ETO leaves a residue on the products and some of its breakdown products have been found to be toxic. Moreover, treatment with ETO is not effective in killing the bacterial spores. It also affects the flavor of the spices (4). Irradiation treatment offers a promising alternative to ETO in improving the quality of spices for the food industry. Several workers (5, 6, 7) found that a dose of 4-5 kGy was sufficient to improve the microbial quality of spices without affecting its orga-

noleptic quality.

The present work reports the results of the study conducted on selected spices to determine the effective dose to decontaminate the product and to determine its keeping quality.

## **MATERIALS AND METHODS**

### **Sample Preparation and Treatment**

Samples of selected spices were supplied by two local food manufactures. The spices included black and white pepper, onion and garlic powder, nutmeg and coriander. Each of the spices was divided into 4 lots (200 grams per pack). One lot was used as controls (non-irradiated) and the other three lots, as test samples (irradiated). The samples were packed in plastic bags of 0.05 mm thickness.

Samples of selected spices were irradiated at doses of 3, 6 and 9 kGy using Cobalt-60 as source of gamma radiation. The exposure to radiation was done in the Gamma Cell 220 at a dose rate of about 25 kGy per hour. The irradiation facility is located at the Philippine Nuclear Research Institute.

### **Microbiological Evaluation**

The total plate count (TPC) and total mold and yeast counts (TMYC) were determined using plate count agar for the pour plate method for TPC and potato dextrose agar for the surface plate method for TMYC. The plates were incubated at 37 degrees centigrade for 48 hours for TPC and 25 degrees centigrade for 3 days for TMYC. Colonies were counted after 2 to 3 days incubation period. Predominant colonies were isolated for both irradiated and non-irradiated samples for identification.

### **Sensory Evaluation**

#### **a.) Triangle Test**

About 1 gram of powdered onion, garlic, nutmeg and coriander were diluted with 350 ml. warm water. The 6 panelists were presented with three coded samples. Two of the codes were the same and the third code was different. The samples were tasted and the coded sample was checked if they are different. The level of significance was determined using statistical table from Appendix C (9).

#### **b.) Pungency Test**

The method involved the aqueous extraction of powdered black and white pepper in 95% ethyl alcohol and diluted with 5% sucrose solution. The test was

performed by 5 panelists to determine the horness pungency of the samples. The scores were ranked according to Schedule B (Scoville Heat Units) of ASTA method.

### Storage Studies

Samples of black and white pepper were stored at ambient temperature for six months to determine the changes in quality of the spices during storage. Monthly evaluation of microbiological, sensory and moisture content were done.

## RESULTS AND DISCUSSIONS

### A. Microbiological Evaluation

The total microbial count among irradiated spices decreased with increasing doses of gamma radiation (Figure 1). The initial load of aerobic microbes in most of the spices ranged from  $10^3$  to  $10^6$  colony forming units (cfu) per gram. A radiation dose of 6 kGy reduced the microbial population by as much as 3 log cycles. Bacteria isolated from agar plates consisted mainly of aerobic spore-formers of *Bacillus* and *Pseudomonas* spp. Coliforms and *Salmonella* spp. were not detected in both irradiated and non-irradiated samples.

The total mold and yeast counts were found to be lower in the irradiated than the non-irradiated samples (Figure 2). A decrease of about 3 log cycles was noted in irradiated spices. Complete elimination of mold and yeast was obtained at a dose of 9 kGy. Molds isolated from selected spices were mainly *Aspergillus*, *Rhizopus* and *Penicillium* spp. Thornly (10) showed that an irradiation dose of 2-6 kGy eliminates these organisms up to seven log cycles.

Irradiation of spices at 6 kGy effectively reduced the microbial load within the acceptable limits of not more than  $1.0 \times 10^5$  organisms per gram in case of total plate count and not more than 100 organisms per gram in case of total mold and yeast counts. Maha (11) reported that an irradiation dose of 5 kGy reduced the microbial load of different spices commonly used in food industry.

### B. Physicochemical Evaluation

The moisture content of the spices ranged from 6.5-10.0%. Black pepper had the highest moisture content while coriander had the least. The standard limit for the moisture content of the spices should not be more than 15%. The data obtained showed that both treatments are within the acceptable limits. (Table II) shows the percentages of volatile oil contents of the irradiated samples were comparable with the non-irradiated lots. Similar results on spices were found by Saul (12) where a gamma irradiation dose of 9 kGy showed no significant effect on moisture and volatile oil contents.

### C. Sensory Evaluation

Based on the triangle tests for sensory evaluation, gamma irradiation did not significantly affect the flavor and the color of the spices were of acceptable quality (Table III). Pungency evaluation for black and white pepper gave satisfactory results at the applied radiation doses.

### Storage Studies

The initial total plate count of black and white pepper was about  $10^6$  cfu per gram. A radiation dose of 6 kGy reduced the population by 3 log cycles (Figure 3). Irradiated black and white pepper, showed a reduction in the total microbial counts during the six month storage period (Figure 4). A similar decrease in mold growth was observed in the black and white pepper during the first month of storage, to  $10^5$  cfu per gram and decreases slightly to  $10^4$  thereafter.

The moisture content of the samples increased slightly during storage however, it did not exceed the standard limit of more than 15% (Table IV).

Apparently, storage time showed no significant effect on the organoleptic qualities of black and white pepper. The overall mean score for the pungency of pepper indicates that the irradiated samples were of acceptable quality (Table V).

## CONCLUSION AND RECOMMENDATIONS

On the basis of the investigations done in different spices, it is concluded that gamma radiation can be effective in decontaminating spices from microorganisms. An irradiation dose of 6 kGy is recommended to decontaminate the samples within the acceptable limit that is not more than  $10^5$  cfu for total microbial counts and not more than  $10^2$  cfu per gram for mold and yeast counts.

The amount of moisture and volatile oil contents in the spices showed no marked differences even up to a dose of 9 kGy. There was no significant difference between the organoleptic qualities of the irradiated and non-irradiated samples. Microbial reduction observed in irradiated black and white pepper was maintained during a storage period of six months.

## REFERENCES:

1. Kiss, I., Farkas, S., et al. Improvement of Food Quality by Irradiation, Proc. of a Panel IAEA, Vienna 157, 1974.
2. Crop Statistics Bulletin Trends and Production, Exports and Prices, Bureau of Agricultural Statistics, Department of Agriculture, 1988.

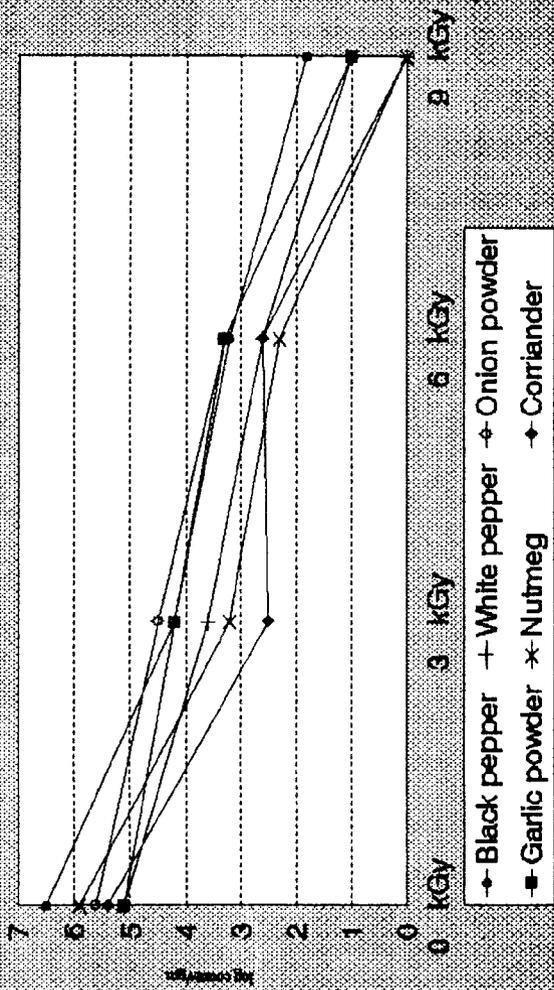
3. Annual Report, Foreign Trade Statistics of the Philippines National Statistics Office, Manila, 1987.
4. International Consultative Group on Food Irradiations (ICGFI). Irradiation of spices, herbs and other vegetable seasonings, IAEA TECBOC - 639, 1992.
5. Saputra, T. S., Sudarman, Harsoyo H. Gamma Irradiation of Spices, Atom Indonesia, Volume 8 No. 2, 1-18, 1982.
6. Maha, M., Purwanto, Z. I., Technology Transfer of Irradiated Spices and Dried Fish, paper presented to FAO/IAEA Seminar for Asia and the Pacific, Shanghai, China April 7-11, 1986.
7. Juri, Muhammad L., Ito, Hitoshi., Distribution of Microfloras in Spices and their Decontamination by Gamma Radiation, paper presented at the FAO/IEAE Seminar, Shanghai, China, April 7-11, 1986.
8. ASTA Analytical Methods, Steam Volatile Oil, Method 5.0 9-11.
9. Gatchalian, Milflora M., Sensory Evaluation Methods with Statistical Analysis, UP Diliman, p.400
10. Thornly, M. J., Radiation Resistance Among Bacteria. *Journal of Applied Bacteriology* 26 (2) 334-345, 1963.
11. Maha, M. Technology Transfer for Irradiation of Spices and Fishery Products in Indonesia, Proc. of Final Research Meeting in Thailand, IAEA, FAO Seminar, Nov. 1988
12. Saul, Christine, S. Irradiation of Spices & Herbs. Proceedings on Commercialization of Ionizing Radiation for Treatment of Food. Australia, April-May 1988.

Table 1. Quantity and value of production, export and import of spices (1988-1993).

Year	Production		Export		Import	
	Quantity (10 <sup>3</sup> metric tons)	Value (P1,000,000)	Quantity (GK)	Value (P1,000,000)	Quantity (GK)	Value (P1,000,000)
1988	25.5	210.7	117681	2451514	772913	22983632
1989	26.3	967.5	84799	3309825	680016	28150550
1990	27.0	1202.7	61401	2617347	850574	28343723
1991	26.5	1454.9	378871	6637349	1157523	34759895
1992	26.8	1494.3	ND	3645850	1078701	38243475
1993	24.4	1452.7	ND	ND	ND	ND

ND = no data

Figure 1. Total plate counts of different spices at varying doses of  $\gamma$ -radiation.



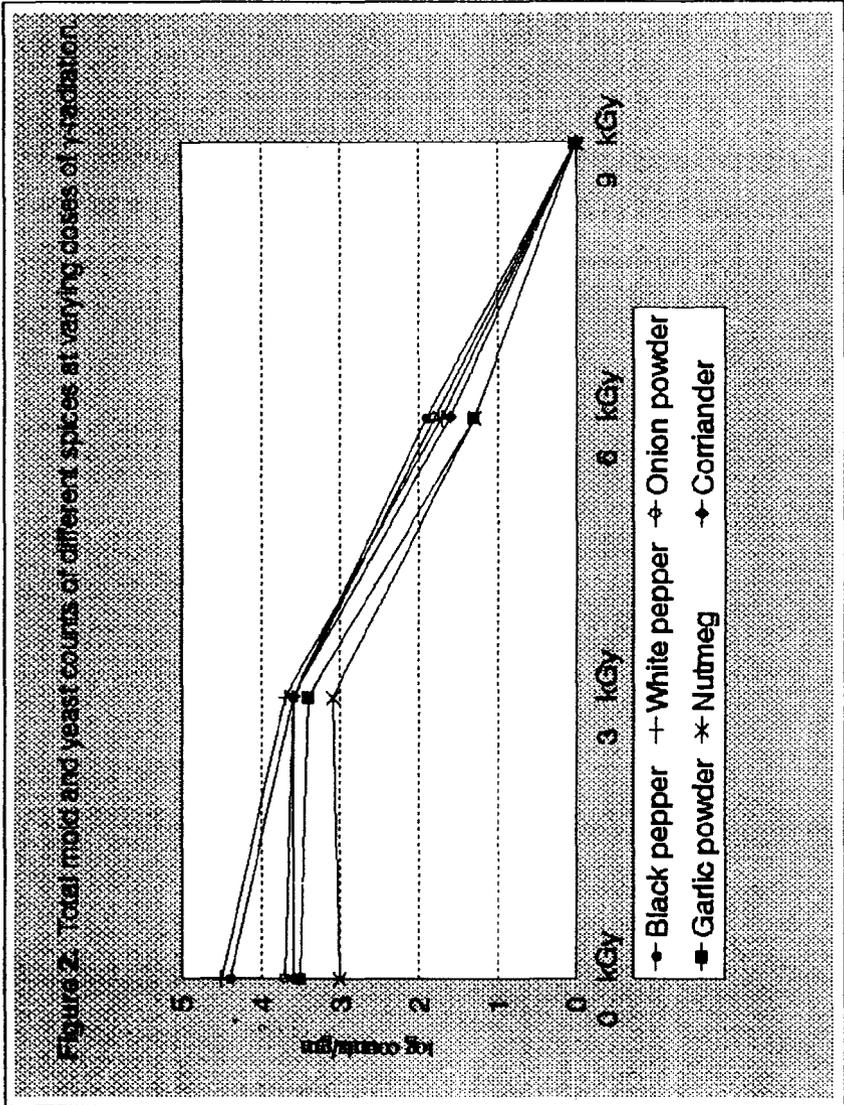


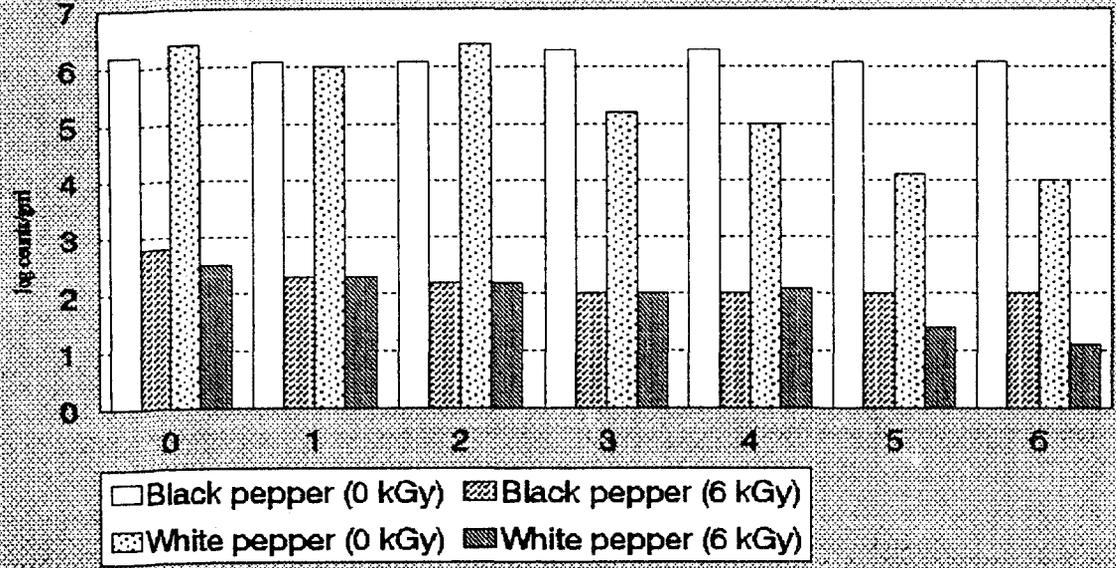
Table 2. Percent moisture and volatile content of non-irradiated and irradiated spices.

Spices	DOSE (kGy)							
	0		3		6		9	
	Volatile oil	Moisture content						
Black pepper	---	10.0	---	9.8	---	10.0	---	9.5
White pepper	---	6.8	---	6.0	---	6.0	---	6.3
Onion powder	---	6.6	---	6.0	---	6.2	---	6.3
Garlic powder	---	7.8	---	7.2	---	7.0	---	7.3
Nutmeg	5.6	6.8	5.0	6.4	5.5	6.2	5.3	6
Coriander	0.6	6.5	0.53	6.0	0.5	6.1	0.6	6

Table 3. Triangle test evaluations of different spices at varying irradiation doses.

<b>Spices</b>	<b>Dose (kGy)</b>	<b>No. of corrected judgements; n=12</b>
<b>onion powder</b>	3	4
	6	3
	9	4
<b>garlic powder</b>	3	5
	6	7
	9	4
<b>nutmeg</b>	3	4
	6	7
	9	5
<b>corriander</b>	3	6
	6	5
	9	4

**Figure 3.** Total plate counts of  $\gamma$ -irradiated (6 kGy) black and white pepper after 6 months storage at ambient conditions.



**Figure 4.** Total mold and yeast counts of  $\gamma$ -irradiated (6kGy) black and white pepper after 6 months storage at ambient conditions.

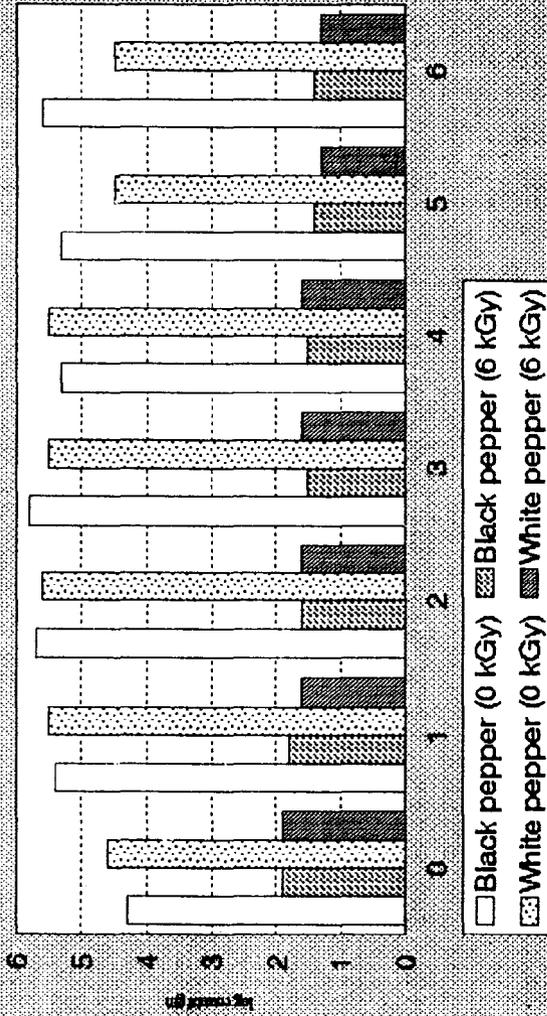


Table 4. Moisture content (%) of black and white pepper stored at ambient conditions for 6 months.

**Moisture content (%)**

	0 month		1 month		2 months		3 months		4 months		5 months		6 months	
	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy
<b>Black pepper</b>	7.00	6.90	6.37	6.56	7.10	6.80	6.80	7.00	7.50	7.40	7.40	7.00	7.00	6.30
<b>White pepper</b>	5.80	6.10	6.31	6.20	6.90	6.40	6.40	6.60	6.70	6.80	6.90	6.70	6.10	5.80

Table 5. Pungency evaluation (s.u.) of black and white pepper stored at ambient conditions for 6 months.

**Pungency (s.u.)**

	0 month		1 month		2 months		3 months		4 months		5 months		6 months	
	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy	0 kGy	6 kGy
<b>Black pepper</b>	16,000	16,000	16,000	16,000	16,000	16,000	16,000	15,000	14,000	14,000	14,000	14,000	14,000	14,000
<b>White pepper</b>	20,000	20,000	18,000	18,000	18,000	18,800	18,000	18,000	18,000	18,000	18,000	18,000	18,000	18,000



# FOOD IRRADIATION IN A DEVELOPING COUNTRY

*Alicia O. Lustre Ph D.*

*Director, Food Development Center, National Food Authority*

## INTRODUCTION

A discussion on Food Irradiation is most appropriate to the theme of this conference. Irradiation has long been established as effective in controlling food postharvest losses and therefore in potentially increasing the volume and quality of available food in a country.

The commercialization of food irradiation technology however is only slowly taking place due to economic and social factors that impose formidable constraints in its adaptation. It is therefore timely that we discuss the status of food irradiation applications at this forum and evaluate the role which this technology can play in addressing the problems of food security in developing countries.

### **Status of the Application of Food Irradiation in Developing Countries.**

Food irradiation is being used in developing countries for the same reason it was first introduced in more advanced countries, which is to control the following quality problems in foods:

- Sprouting and other physiological processes in the storage of tubers and bulbs
- Presence of high levels of bacteria and/or bacterial pathogens in animal foods (as frozen shrimps and fermented meats) and in dried foods ( as spices, seasonings).
- Presence of insects as fruit flies and the seed weevil in mangoes and insect contaminants in dried fish and other dried products susceptible to insect infestation on storage.

The objective of irradiation in the above is to improve the physical and/or hygienic quality of food as well as to reduce its rate of deterioration during storage.

Many developing countries have been assisted by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in research and development and in the transfer of food irradiation technology to the local industry , nine countries in Asia and the Pacific, six countries in Latin America and the Caribbean, five countries in Europe and the Middle East and eight countries in

Africa.

Except for countries in Africa where only research and development activities on irradiation are taking place, several of the above developing countries have regulatory permits and demonstration facilities for food irradiation.

The progress of food irradiation in developing countries vary. In China there are 39 irradiation centers capable of conducting food irradiation in pilot/commercial scale. In the Republic of Korea, a facility used for food irradiation, is operated as a private company with technical support from the Korea Atomic Energy Institute. A plant for the irradiation of potatoes has been in operation in Japan for 20 years. In other countries as India, Indonesia, Thailand, and Vietnam, there are demonstration facilities which have been used to test market irradiated foods and/or to provide irradiation services to the local industry. In the Philippines we have a pilot multipurpose irradiation plant.

The recently concluded Joint FAO/IAEA Asian Regional Project on Food Irradiation with Emphasis on Process Control and Acceptance (RPF1-Phase III) established conditions for the potential large scale irradiation of food products of interest to the participating countries. The foods investigated in this regional project and the participating countries were the following:

1. Spices: China, India, Indonesia, Korea, Pakistan, Sri Lanka,
2. Dried Fish: Bangladesh, Korea, Vietnam
3. Root Vegetables: (Potatoes, Onions and/or Garlic) Bangladesh, China, Japan, Pakistan, Vietnam
4. Cereals (rice) and Pulses (mungbean) Bangladesh, Indonesia, Thailand, Vietnam
5. Frozen Seafood: Korea, Japan, Pakistan, Philippines, Thailand, Vietnam
6. Fresh Mangoes: China, India, Philippines, Sri Lanka

The above Project established parameters for the pre-irradiation, irradiation and post irradiation handling of irradiated foods. This information is important to the eventual transfer of the technology to the industry.

Work in India for example on the irradiation of mangoes as a quarantine treatment against the mango seed weevil has shown that irradiation does not cause complete mortality of adults at doses of 0.30 to 0.75 kGy or even as high as 2 kGy. A recommendation has been made to irradiate the fruit at the hard green stage when the level of adults is less and to change the criterion presently used for quarantine security, from mortality to inability to produce viable offspring, for the mango seed weevil.

Extensive investigations have been conducted on the use of irradiation to reduce the microbiological load in spices. Studies confirm that irradiation can be used to upgrade hygienic quality without affecting the sensory characteristics of the many different types of spices investigated. The ban imposed by some countries in the use of ethylene oxide to decontaminate spices, has strengthened the need to use irradiation for the treatment of this commodity. Spices and dried

vegetable seasonings for example have become the most common products irradiated in Korea since the government banned ethylene oxide fumigation of foods on July 1, 1991.

Another food item where irradiation is necessary is in the Thai traditional fermented raw pork sausage known as Nham. Irradiation of the product to reduce microbiological contamination is necessary because the product is eaten raw. The Thai Irradiation Center irradiated about 44 tons of Nham over an 8 month period in 1993 and expects to irradiate about 4,800 kg of Nham per month for the local food industry.

Large scale studies on the irradiation of dried fish, mung beans, onions and garlic of staple grains such as rice, have all proven technically feasible and acceptable to consumers in the Asia Pacific countries involved in this coordinated research program.

### **Food Irradiation in the Philippines**

The application of food irradiation in the Philippines as in most developing countries, stems from the following needs.

- the need to reduce postharvest losses in the storage and marketing of perishable foods.
- the need for technology to meet the phytosanitary and microbiological requirements for the export of food products

Unavoidable postharvest losses in the transport of fruits and vegetables, per our experience at the Food Development Center can range from 20% - 60%. The distances between producing and consuming areas and the poor infrastructure for transport and storage contribute to the high level of losses.

The export of foods is important to the Philippine economy. Frozen prawns, desiccated coconut and fresh mangoes are among the country's top food exports and contribute about 218 million (or 25% of total food exports), 60 million and 18 million US dollars respectively, to the value of total exports. Stringent requirements for the microbiological quality of seafood and desiccated coconut have caused rejection of these products in the export market.

The first publication on food irradiation in the Philippines in 1969, was on the effects of low dose irradiation in preventing sprouting of onions and ginger and extending the shelf-life of peanuts. Since then a total of nine (9) food products have been studied for possible application of gamma irradiation in the control of quality. These include; bananas, for the control of rotting; mangoes, for the destruction of fruit fly, onions, for the control of sprouting; garlic, for the control of sprouting; coffee, for the control of the coffee bean weevil; copra, for the control of the copra beetle; desiccated coconut, for the elimination of salmonella; dried and smoked fish for the control of insects and the extension of shelf-

life; and flour, for the destruction of the red flour beetle. Wholesomeness testing on irradiated fresh mangoes and smoked fish have also been carried out and showed that neither product exhibit mutagenic effects attributable to the irradiation treatment.

Recent studies on food irradiation in the Philippines have centered on the established of procedures for the quarantine treatment and ripening control of fresh mangoes, the elimination of *Salmonella* in pond-grown prawns and the test marketing of irradiated onions, garlic and spices.

a. Onions and Garlic

Test marketing trials on onions showed that irradiation resulted in a 30 to 40% reduction in storage losses, 20% reduction in in-transit losses from Luzon to Mindanao and a 28% reduction in losses during retail sale. For garlic, the major benefit of irradiation was the control of sprouting during low temperature storage, which in turn allowed storage of garlic with minimal weight loss and insect infestation for a period of one year.

b. Mangoes

Irradiation of mangoes as a quarantine treatment for fruit flies was investigated by the Philippine Nuclear Research Institute to provide a substitute for ethylene dibromide which was banned in the US and Japanese markets. Irradiation at a dose level of 100 Gy was established in 1990 as adequate for the destruction of fruit flies by Manoto. At this dose level. Lizada et al. found that there was no inhibition in the overall ripening of the fruit. Irradiation of mangoes as a quarantine treatment against fruit flies however has assumed less importance because vapor heat treatment, which has been successfully commercialized in the Philippines, is the approved quarantine treatment for fruit flies in the Japanese market.

Another pest of quarantine significance in Philippines is the mango seed weevil. A survey is presently being conducted by the Philippine Department of Agriculture with the United States Department of Agriculture, to confirm the presence of the seed weevil in Philippine mangoes. If present, the seed weevil can only be reached by irradiation and a quarantine protocol based on irradiation of this pest, will therefore have to be established. It not verified as present in Philippine mangoes however (as indicated survey results recently completed for Guimaras island), irradiation will no longer be necessary as a quarantine treatment for mangoes.

c. Shrimps

Because of its role in helping the industry avoid products rejections in the export market the Food Development Center has conducted several studies to find a practical solution to the problem of the unavoidable presence of *salmo-*

*nella* in pond grown shrimps. As in several other seafood produced by aquaculture farming, salmonella which is not present in marine shrimps, has been found by FDC in a survey of 100 shrimp ponds all over the country, to be a natural contaminant of the pond. The pathogen survives normal good manufacturing procedures for the freezing of prawns.

Studies on irradiation of frozen shrimps at FDC have established some important conditions for the irradiation of prawns. The D value for the destruction of *S. velterverden*, the specie being isolated in Philippine shrimps in the international market, was 0.58 kGy, and the required dose level for the destruction of salmonella, without the development of an intense smokey odor and flavor was 1.2 kGy.

The studies showed an unusual sensitivity of Philippine prawns to develop a smokey odor and flavor on exposure to irradiation, the intensity of the flavor increasing with increasing dose. Because of this, it is important to control the irradiation dose at the level of 1.2 kGy and to insure that product temperature during irradiation is retained at -18°C. The oxidation of sulfur containing amino acids, which has been reported as responsible for the development of the smokey odor and flavor in shrimps.

### **Commercialization of Food Irradiation in the Philippines**

The beneficial effects of irradiation on food quality will not by itself lead to the commercial adaptation of the technology if the marketability and profitability of the product are not assured.

In the control of sprouting in onions for example, the preferential purchase by the consumer of irradiated onions and the cost of irradiation, will be the major factors that will influence the adaptation of the process by private industry. The reduction in storage losses due to irradiation will be unimportant unless it is accompanied by an increase in the profitability of the product.

Marketability and profitability affect the commercialization of any new food process. There are problems unique to food irradiation however that affect private sector interest in the technology. These problems are the following:

#### **1. Acceptance of irradiated foods by regulatory agencies**

The marketing of any food product is governed by regulations that insure minimum risk to public health and the protection of the consumer against fraud and unfair trade practices. Irradiated food products are subject to the same regulations.

Codes of Good Irradiation Practice have been prepared by the International Consultative Group on Food Irradiation (ICGFI) for prepackaged meat and poultry, for the control of pathogens and other microbial flora in spices, herbs and other vegetable seasonings, for the shelf-life extension of bananas, mangoes and papayas, for sprout inhibition of bulb and tuber crops, for insect disinfes-

tation of dried fish and salted and dried fish, for the control of microflora in fish, frog legs and shrimps.

In the Philippines, permits have been given for the market testing of potatoes, onions, and garlic. The preparation of regulations for Food Irradiation has been discussed by parties interested in the irradiation of food commodities with the Bureau of Food and Drug of the Department of Health and is being considered.

The regulatory impediment to the commercialization of food irradiation however comes from the non-acceptance of the process by certain countries. Although a Joint Expert Committee on Food Irradiation of the World Health Organization declared in 1981 that food treated with ionizing radiation up to doses of 10 kGy is safe, the irradiation of foods is not accepted in all countries. It is for example banned in New Zealand, temporarily banned in Australia, and not allowed in Malaysia. Where it is accepted as in the United States, irradiation of foods is subjected to approval on a product by product and purpose basis. It is allowed in frozen poultry for treatment against *Salmonella* in pork, for destruction of trichinella, in spices for the reduction of the microbial load. The use of irradiation as a quarantine treatment has been allowed only for papayas exported to the US mainland from Hawaii.

The apparent reluctance by export markets to accept irradiation as a safe process for food preservation hampers international trade of irradiated foods. This has important repercussions for developing countries as irradiation of commodities for export is expected to add to the volume throughputs and financial profit of a multipurpose irradiation plant.

Traditional food processing technologies as freezing, canning, smoking sugar preservation and salting have been used by man for many years to preserve his food. Even if new science and technology point to health hazards in such processes now, regulatory agencies have enough expertise and practical experience with the risks involved, if any, with traditional food processing technologies. The lack of a milar history of safe consumption for irradiated foods, is probably responsible for the slow acceptance of the process by regulatory agencies.

### **Acceptance by the Consumer**

For countries that have started to market food in commercial scale, consumer acceptance has been a major drawback in the progress of food irradiation. In Korea for example, it is the most important problem in the operationalization of a commercial multipurpose irradiation plant. In Japan, consumer activism stopped developments in the commercial irradiation of potatoes.

In the Philippines, we have not experienced fierce consumer activism or opposition to the test marketing of irradiated onions and garlic.

There are three types of consumers in the market for irradiated foods. The first are the antinuclear activists who oppose anything nuclear. The second is the layman who associates food irradiation with the destructive effects of radioactiv-

ity. The third and perhaps the bigger group is the layman who may or may not be aware of what food irradiation is, but who will always choose the better quality food irrespective of whether it is irradiated or not.

Antinuclear activists refuse to accept irradiated foods and have been part of an advocacy campaign to discourage its acceptance by the consumer. The other types of consumers can be reached through proper education and the conduct of a valid and believable information campaign.

In a survey of 700 consumers in Korea, a high 72% of the general public was aware of food irradiation processing. Of the total consumers surveyed, 37% were ready to buy irradiated foods, 51% requested for more information, 5% would not but it outright and 6% would follow the advise of the Consumer Union. This survey highlights the great importance of consumer education in the introduction of irradiated foods into the market.

### **Cost Factors in Food irradiation**

The capital investment for a food irradiation plant is high. It has been reported (May, 1993) that the cost of a semiautomatic irradiator for food processing, with an initial source strength of 100 kCi and essential equipment, is approximately 800,000 US dollars. In addition, biological shielding of the irradiator will cost 500,000 US dollars or a total amount of 1,300,000 US dollars.

The above cost of a food irradiation plant for example is almost equivalent to that of vapor heat treatment plants built in the country in 1987 to replace ethylene dibromide fumigation in the quarantine control of fresh mangoes exported to Japan. In this case the capital investment in the new technology although high, was readily made by the industry because it was essential to keeping an existing profitable market open.

Preliminary feasibility analysis conducted by FDC indicates that irradiation of onions and garlic, by minimizing distribution and marketing losses, will be a profitable operation. In spite of this, the industry will not easily risk an investment in an onion and garlic irradiator because present practices for the storage and distribution of these commodities, in spite of the losses incurred, are still profitable.

The continuous availability of food products for irradiation is important to the profitable operation of a food irradiation plant. This is not easy in a developing country like the Philippines as agriculture is not concentrated in large farms and the transport infrastructure is not always reliable. Further, in the last 6 months of the year, unexpected typhoons will make it difficult to insure the timely arrival of food raw materials.

Food processors know only too well, that there will be days when the arrival of raw materials will be higher than the plant capacity and days when the plant will be idle for failure of expected commodities to arrive. The lack of throughput will increase the cost of operation and the long waiting times may

reduce the quality of products as onions and garlic, that have to be irradiated within a specified postharvest period.

The benefits expected from the commercialization of food irradiation are better established due to many pilot scale trials on the test marketing of various food commodities that have taken place in different countries. The problems are also better identified. The reevaluation of the benefits relative to the problems should help paint a better picture of the role irradiation can play in increasing the availability and supply of good quality food in the country.

### CONCLUSIONS

1. Food irradiation is essential to upgrading the quality of basic food commodities in the domestic market. By extending shelf life and reducing storage losses, the supply of these commodities will be insured throughout the year at lower prices.
2. Consumer education is necessary to create understanding of the benefits of food irradiation in order to facilitate its acceptance in the domestic market.
3. Private sector will not readily invest in a food irradiation plant due to its high capital cost. Moreover, unlike the requirement for the vapor heat treatment of mangoes in the Japanese market, there is no compelling need for entrepreneurs to invest in food irradiation to continue selling their products in the local market.

### RECOMMENDATIONS:

1. Specific studies have to be conducted to evaluate the cost/benefit of food irradiation in the light of present applications, problems and needs. These studies should focus on potential applications that have not been evaluated as the irradiation of rice and other cereals and grains which are widely consumed in large quantities. Moreover, the feasibility study should be based on actual volumes and locations of commodities to be irradiated including non-food products, needed to meet through put requirements for the operation of the plant.
2. An estimate of the magnitude of the existing consumer risk from the consumption of foods treated by chemicals and/or infested or contaminated during storage should be made in order to put the importance of investing in food irradiation in proper perspective.
3. Government should play a strong role in funding the transfer of technology to the industry for the following reasons:

- 4.1 Agro-business entrepreneurs seldom have the trading experience to source and market the total combination of food commodities needed to keep an irradiation plant in full operation. For this reason, entrepreneurs presently prefer to use a service irradiation facility rather than to own one.
- 4.2 In view of the problems unique to the marketing of irradiated foods, private industry is not ready to take a risk in putting up the capital investment needed in the establishment of a food irradiation plant.
5. The parameters for the irradiation of products for the export market as shrimps, desiccated coconuts and others should continue, to be established because concepts regarding food safety and quality change. Food irradiation may thus still be accepted as a substitute for harmful chemicals and as an assurance against the presence of pathogens in foods.

#### **ACKNOWLEDGMENT**

I would like to thank the organizers of this conference for giving me the opportunity to discuss food irradiation in this forum.



# LUMINOUS BACTERIAL PRODUCT: A POTENTIAL INDUSTRIAL FOOD DYE

*Prima C. Ragudo-Franco and Kenneth H. Nealson*  
*Department of Biology, College of Arts and Sciences*  
*Mariano Marcos State University, Batac, Ilocos Norte, Philippines*

## ABSTRACT

Recombinant DNA Technology plays an important role in the recovery of natural products. This technique was employed in *Xenorhabdus luminiscens* strain Hm primary, a luminous bacterium to recover its pigment which has the potential to be used as a dye in the food industry.

This paper involves studies about the genetics of pigment formation. The pigment genes were cloned and expressed in *Escherichia coli*; the recombinant plasmid named pCGLS 100 was screened and isolated, and a partial restriction map of the 8.1 kb insert was constructed. This fragment was nick translated and subsequently used as a probe for other *Xenorhabdus* species. The pigment of the recombinants was extracted and characterized.

## INTRODUCTION

Natural additives is gaining predominance over synthetics in the food industry. The quest for natural products to replace currently used synthetic food colors is a major concern of food scientists. Recombinant DNA technology is an important technique used in the recovery of natural products. This technique was employed in cloning the red pigment genes of *Xenorhabdus luminiscens* strain Hm primary in *Escherichia coli*.

The bacterium *Xenorhabdus luminiscens* is a Gram negative rod belonging to the family Enterobacteriaceae (24,11,4). Its life cycle (Figure 1) includes a phase as symbiont, residing in the intestines of its nematode host *Heterorhabditis bacteriophora*. This harmonious association is a potent pathogen for a wide variety of insects. The infective nematode carrying *X. luminiscens* penetrates the insect larva via gut, mouth, spiracles trachea(23). Once the nematode reaches the insect hemolymph, it releases its bacterial symbionts. The bacteria then multiply and cause death to the insect(10). The insect carcass provide the nutrients for the reproduction of the nematode, which then completes its life cycle giving rise to infective juveniles harboring *X. luminiscens*. The infective juveniles emerge from the insect's carcass ready for the next susceptible host(17). The insect cadaver which does not putrefy immediately because of the antibiotics produced by the bacteria(1), becomes luminous due to the light produced by the bacteria(23) and then develops a deep red color(14) due to the pigment produced by *X. luminiscens*.

In vitro, it has been shown that *X. luminescens* Hm primary gives rise to secondary variants after a long period of incubation (5). *Y.luminescens* Hm primary have characteristic luminosity (3,5,9,8). It can produce extracellular products such as antibiotics (1.16) protease (23), lipase (2) and a red pigment (21,5,23). It also takes up neutral dye. Such characteristics are not present in the secondary form.

This study focuses on the genetic aspects of pigmentation in *X. luminescens*. It presents the screening of a pigment clone from its genomic library, the construction of a partial restriction map of the insert, the probing of other *Xenorhabdus* strains using the <sup>32</sup>P labeled putative pigment genes and the characterization of its product. Since the pigment from *X. luminescens* has been characterized to be an anthraquinone (Figure 2) (21) and since anthraquinone is known to possess some dyeing qualities, cloning and expression of the pigment genes of this bacterium may offer the chance to explore its potential as an inexpensive alternative red dye in the food industry.

## MATERIALS AND METHODS

### I. Library Construction

The genomic library from which the pigment clone was screened was constructed by Dr. Susan Frackman at the Center for Great Lakes Studies, University of Wisconsin at Milwaukee, USA.

The genomic DNA of *X. luminescens* Hm primary was digested with *Sau* 3A, ligated to the *Bam* H1 site of pUC 18, and was transformed in *E. coli*, as diagrammed in Figure 3.

### II. Growth Medium

Throughout the course of the experiment, unless otherwise indicated, the bacteria were grown and maintained on LB which consists of: 5g of yeast extract, 5g of NaCl, 10g of bactopectone (Difco) in 1 liter of distilled water. Ampicillin (Ap) was added to a final concentration of 100ug/ml. For solid medium, 15g of agar was added per liter.

### III. Screening

In order to avoid confluent growth and ensure proper identification of the clone, the dilution factor yielding approximately 100 CFU was determined prior to screening. To each of the 150 plates used, 100ul of the inoculum was dispensed and spread on LB+Ap plates which were inverted and incubated overnight at 30°C in a New Brunswick Controlled Environmental Shaker. All the plates

were plated in replica to the same media using replica plate pads (Repliplate FMC Bioproducts) and were incubated for 4 days under the same conditions. After the end of the incubation period, each of the plates was sprayed with 1N NaOH. This assay was based on the finding (21) that the pigment is pH sensitive; yellow at pH 9 and red at pH 9.5. One colony turned red in response to the NaOH; it was matched with the colony from the master plate, and scored as the red pigment clone. The clone was maintained in LB+Ap with 25% glycerol in the -70 C freezer.

#### IV. Plasmid Isolation

Large scale plasmid isolation (alkaline-lysis method) was done (13). The recombinant plasmid (pUC 18+ the pigment genes of *X. luminescens* Hm primary) was named pCGLS 100.

#### V. Restriction Mapping

Restriction endonucleases recognizing 6 bases were chosen to digest 1 ug of pCGLS 100 (Table 1). Single and double digestions were performed according to the conditions recommended by the supplier of the enzymes (BRL). Plasmids and restriction fragments were analyzed by 0.7% gel electrophoresis in Tris Borate buffer (TBE) using 1 kb ladder molecular size as a standard (13).

#### V. Preparation of probe from pCGLS 100

100 ug of pCGLS 100 was digested with Pst 1 and Sst 1 for 1 hour at 37 C. The digestion was ran overnight in a 1% agarose gel and the 8.1 kb insert was cut from the gel after viewing it in UV light. This was frozen, thawed, extracted in phenol-chloroform and precipitated in ethanol (13).

The insert was nick translated using BRA nick translation kit. The reaction: 2 uL of the insert DNA; 5 uL of 10uCi of <sup>32</sup>P; 5 uL of dNTP; 33uL of water and 5 uL of the enzymes was incubated at 15 C for 1 hour. At the end of the incubation period, 5 uL of the stop solution was added and the mixture was loaded onto a spin column (Sephadex). This was spun in a clinical centrifuge at high speed for 6 minutes. Its activity was counted in a scintillation counter (Tri-carb Liquid Scintillation Analyzer Packard) using 10 uL of the probe placed in a milliliter of scintillation cocktail (Complete counting cocktail 3a70B, Research Products International Corp.). The probe was then denatured by boiling for 5 minutes. VII. Genomic DNA Isolation

The genomic DNA of the *Xenorhabdus* strains (Table 2) were isolated (13). Some had been previously isolated and kindly provided by Mike Anhalt and Barb Wimpee.

### VIII. Southern Blotting

A ten microgram sample of each genomic DNA (with pCGLS 100 as the control) was digested with 30 U of Eco R1 at 37°C overnight in 0.7% agarose gel in TBE at 25 volts). The gel was stained with ethidium bromide and photographed (13). The DNAs were then transferred to Nytran paper filter using a Vacuum Blotting System LKB 2016 {Vacu Gene Pharmacia LKB Biotechnology}.

Nytran was first soaked in sodium chloride sodium citrate (SSC) and was set with a gel in a blotter. The vacuum was set at 45 cm. water and the gel was covered with depurination solution for 3 minutes after which it was removed. The gel was then sandwiched in between two pieces of Whatman #0.3 filter paper and baked for 2 hours at 80°C in a vacuum oven. This procedure, referred to as a Southern Blot, was based on the instructions of the manufacturer.

### IX. Prehybridization and Hybridization

The Southern Blot prepared was placed in a bag of 5 ml prehybridization solution composed of 50% formamide, 2.5 X Denhardt's solution, 10 mg/ml salmon sperm DNA, 0.1% Sodium dodecyl sulfate (SDS) and 5 X SSC which nonspecifically saturates the single or double stranded DNA bound to the Nytran filter (13). This was allowed to prehybridize for 3 hours at room temperature. The prehybridization solution was squeezed out and 10 ml of the hybridization solution was added and allowed to hybridize overnight with gentle agitation at room temperature. This was a modification of the protocol given by the Nytran manufacturer.

### X. Washing

The filter was removed from the bag and transferred to a plastic dish with 100 ml of 6 X SSC and 0.1% SDS for 1 minute, and then allowed to dry.

### XI. Autoradiography

The filter was covered with Saran wrap, inserted with Kodak film in One Source Cassette Box, and placed at -70°C for 4 days. The filter was then developed and hybridization were visually checked.

### XII. Pigment Isolation

The attempts to extract the red pigment from the recombinant *E. coli* in ethyl acetate (21) were not successful. A wide variety of solvents (Carbon tetrachloride, cyclohexane, hexanes, isopropyl, acetone, toluene and acetonitrile) were tried at various pH (adjusted by adding HCl and NaOH).

Alternatively, the pigment was concentrated by freeze drying. X.

*luminescens* strain Hm secondary, *E. coli* + pUC 18 and the recombinant *E. coli* containing the putative pigment genes were grown overnight with shaking in test tubes with 5 ml of LB+Ap at 30C. One ml of each of the cultures was inoculated into separate flasks of 500 ml of LB+Ap and grown overnight in 30C with shaking. At the end of the incubation period, the cultures were transferred to centrifuge bottles and spun for 10 minutes at 5000 X g in a Beckman Centrifuge (Model J-21). The supernatant of each was poured into a lyophilization bottle which was then immersed and rotated in a bucket filled with dry ice and methanol until all of the liquid was frozen at the sides. This was then lyophilized in a Labconco Freeze dryer for two days. Unfortunately, the preparation liquefied and even after longer period of exposure, the sample did not dry. This was circumvented in the next trial by growing the organisms in LB without NaCl. The above-mentioned procedure was followed except that the NaCl was eliminated. After the samples dried, they were gathered in plastic tubes, capped and stored in a box of silica gel. The samples were sent to Dr. William Fenical at the Scripps Institution of Oceanography in La Jolla, California.

## RESULTS

### I. Screening

A total of 10,250 colonies were screened and only one turned red in response to the 1N NaOH. This was scored as a red pigment clone.

### II. Restriction Mapping

Hexameric restriction endonucleases with and without known sites in the vector pUC 18 were used to digest the recombinant plasmid pCGLS 100 (Table 1). Both single and double digestions were employed to determine the restriction sites as well as the total size of the insert. With single digestion, Cla I, HindIII,

Pst I, Nhe I, Nru I and Hpa I cut the plasmid once, Eco RV and Ava I twice and Eco R1 thrice. Since Pst I and Sst I were found to have no sites at all, and since these exist at the multiple cloning site of the vector, they were designated as site references for the ends of the insert. Double digestions using the two enzymes revealed an 8.1 kb insert (Figure 4). From Sst I site, the following are the restriction sites of the enzymes used: Cla I, 0.45 kb; Nru I, 2.3 kb; HpaI, 5.4 kb; Nhe I 1.2kb; EcoR1, 3.4 kb and 6.4 kb; Eco Rv, 0.5 kb and .8 kb (Figures 5a and 5b). The recombinant plasmid pCGLS 100 contains the 8.1 kb insert and pUC 18 vector as diagrammed in Figure 5b.

### III. DNA-DNA Hybridization

The genomic DNA of the different *Xenorhabdus* strains (Table 2) were di-

gested with Eco R1 and hybridized with the 8.1 kb insert of pCGLS 100 containing the putative pigment genes from *X. luminescens* Hm primary. This 32P labeled DNA hybridized with 7kb, 2.8 kb and 1.8 kb of both *X. luminescens* Hm primary and secondary; 1.1 kb, 2.2 kb, 2.8 kb and 5.4 kb of *X. luminescens* NC19 primary and secondary with and without pigment, 2.4 kb, 3.8 kb and 5kb of *X. luminescens* Fla primary and with 2 two 3.4 kb and 3.9 kb of the control pCGLS 100 (Figure 6). No hybridizations were observed with *X. luminescens* JWH, *X. luminescens* 22A, *X. nematophilus* and *X. poinarii*. This gives information in the distribution of these genes among *Xenorhabdus* species.

#### IV. Pigment Identification

Thus far, it is clear that no anthraquinone was observed, although smaller compounds suspected to be intermediate compounds to the anthraquinone metabolic pathway were detected.

### DISCUSSION

Pigment production by this bacterium has been hypothesized to play a role in attracting prey for the nematodes (20), which is consistent with the fact that pigment production occurs late in the bacterial growth cycle. The pigment is produced at the mid-to-late logarithmic phase of growth (20), and is not visually apparent until the 5th or 6th day of incubation. The same holds true for the bacteria inside the insect carcass (19); red coloration is visible late in infection, at a time when nematodes would be emerging in search of new prey.

Grimont, et. al., (14) have employed pigment production and coloration as taxonomic tools. Since the pigment is pH sensitive, it is crucial that the colonies be maintained in the same pH while pigmentation is considered as a taxonomic basis (20). Even with this addition however, pigmentation is still a qualitative character. A more definitive method might be identification of the genes coding for pigment production. Having cloned the pigment genes for *X. luminescens* Hm primary, it was possible to scrutinize the universality of the genes in other *Xenorhabdus* species. Using Southern blotting, nick translation and hybridization techniques, it was found that the pigment genes from *X. luminescens* Hm primary hybridized with itself, with *X. luminescens* Hm secondary, with *X. luminescens* NC19 primary and secondary with and without pigment and with *X. luminescens* Fla primary (Figure 6). No hybridizations were observed with *X. luminescens* JWH, 22A, *X. nematophilus* and *X. poinarii*. With further refinements, it may be worthwhile considering this as a taxonomic tool, perhaps even a quantitative one.

It was further observed that the same bands from both the primary and secondary forms hybridized with the probe (Figure 6). This shows that the pigment genes have remained the same in the two forms even though the development of the color is different. This supports the notion that the secondary variants are not

simply contaminants. Hurlbert et.al., (16) have suggested that phenotypic switching in *X. luminescens* RH/1 may be attributed to the actions of transposons or insertion sequences. No observations from this study however, support this idea; rather our results suggest that no major gene rearrangements have occurred (although more analyses are needed with larger DNA fragments to establish this with certainty). This further suggests that the sources of the dye are varied and are naturally produced by nearly all strains of *Xenorhabdus*.

Consistent with this is the fact that on all media tested, the secondary form grew faster than the primary (2), even though the primary form is the observed form transmitted into the new prey by the infective nematode (25). If secondary variants are used for infections, the nematode is ineffective in reproduction, suggesting that the nematode must somehow select for the primary forms. These primary forms, then may be important for many purposes, such as the production of antibiotics to temporarily preserve the insect carcass, production of pigment and light to attract prey for the nematode during daytime and nighttime respectively, and the production of other secondary metabolites to perform other functions which are not yet understood.

### CONCLUSION

On the basis of our extraction results, it seems likely that not all of the pigment genes were cloned. This may be the reason behind the solubility differences of the recombinant's product from that of *X. luminescens* Hm primary pigment. Since our insert is only 8.1 kb, we might expect that it contains less than the total information needed. Further work on larger fragments of DNA to possibly isolate the entire set of the pigment genes by using a cosmid should be the next step.

However, in as much as the pigment has been produced at a tremendous rate, its possibility of being used as an inexpensive red dye and preservative in the food industry invites food scientists to work on it. This will then offer an inexpensive alternative to the hard-to-procure red dye which could only be obtained from the beetles in Peru.

### BIBLIOGRAPHY:

1. Akhurst, R. J. Antibiotic activity of *Xenorhabdus* species, bacteria symbiotically associated with insect pathogenic nematodes of families Heterorhabditidae and Steirnermatidae. *Journal of General Microbiology*, 128, 3061, 1982.
2. Akhurst, R. J. *Neoaplectana* species: specificity of association with bacteria of the genus *Xenorhabdus*. *Experimental Parasitology*, 55, 510, 1983.
3. Akhurst, R. J. Taxonomic study of *Xenorhabdus*, a genus of bacteria symbiotically associated with insect pathogenic nematodes.

- cally associated with insect pathogenic nematodes. *International Journal of Systematic Bacteriology*, 34, 38, 1983.
4. Akhurst, R. J. and N. E. Boemare. A numerical taxonomic study of the genus *Xenorhabdus* (Enterobacteriaceae) and proposed elevation of the subspecies *X. nematophilus* to species. *Journal of General Microbiology*, 134, 1835, 1988.
  5. Bleakley, B. and K. H. Nealon. Characterization of primary and secondary forms of *X. luminescens* strain Hm. *FEMS Microbial Ecology*, 53, 241, 1988.
  6. Bowen, D. J. and G. C. Ensign. Intracellular protein crystal of the insect pathogen *X. luminescens*. *Proceedings: Annual Meeting of the American Society for Microbiology*, 1-66, p. 183, 1987.
  7. Couche, G. A., P. R. Lehrbach, R. G. Forage, G. C. Coonry, D. R. Smith and R. P. Gregson. Occurrence of intracellular inclusions and plasmids in *Xenorhabdus* spp. *Journal of General Microbiology*, 133, 967, 1987.
  8. Frackman S. and K. H. Nealon. The bioluminescence genes of *X. luminescens*. *Annual Meeting of the American Society for Microbiology*, 1988a.
  9. Frackman, S., P. C. Ragudo and K. H. Nealon. Bioluminescence and pigment genes of *X. luminescens*. *Annual Meeting of the American Society of Microbiology*, 1989.
  10. Gaugler, R. Biological control potential of Neoplectanid nematode. *Journal of Nematology*, 13, 599, 1988.
  11. Grimont, P. D., A. G. Steigenwalt, N. E. Boemare, W. Hickman- Brenner, C. Deva, F. Grimont and D. J. Brenner. Deoxyribonucleic acid relatedness and phenotypic study of the genus *Xenorhabdus*. *International Journal of Bacteriology*, 34, 378, 1984.
  12. Hurlbert, R. E., J. Xu and C. Small. Colonial and cellular polymorphism in *X. luminescens*. *Applied Environmental Microbiology*, 55, 1136, 1989.
  13. Maniatis, T. E., F. Fritsch and J. Sambrook. *Molecular cloning: a laboratory manual*. Cold Spring Laboratory, Cold Spring Harbor, N.Y. 1982.
  14. Morris, O. N. Susceptibility of 31 species of agricultural insect pests of the entomogenous nematodes *Steirnernema feltiae* and *Heterorhabditis*

*bacteriophora*. 1985

15. Neelson, K. H., T. M. Schmidt and B. Bleakley. Physiology and biochemistry of *Xenorhabdus* (in press) In: Gaugler, R. and Kaya, H. (eds) Entomopathogenic nematodes in biological control. CRC Press, Boca, Raton, U.S.A., 1989.
16. Paul, V.J., S. Frautschy, W. Fenical and K. H. Neelson. Isolation and structure assessment of several new antimicrobial compounds from the insect symbiotic bacteria *Xenorhabdus* spp. *Journal of Chemical Ecology*, 7, 589, 1981.
17. Poinar, G.O. The Natural History of Nematodes. Prentice Hall, Englewood, Cliffs, Ca. 1983.
18. Poinar, G. O. and G. M. Thomas. The nature of *Achromobacter nematophilus* Poinar and Thomas (*Achromobacteriaceae* sp. *Steinernematidae*). *Parasitology*. 56, 385, 1966.
19. Poinar, G. O., G. Thomas, M. Haygood and K.H. Neelson. Growth and luminescens of the symbiotic bacteria associated with the terrestrial nematode *Heterorhabditis bacteriophora*. *Soil Biological Biochemistry*., 12, 5, 1980.
20. Poinar, G.O., R. T. Hess, W. Lanier, S. Kinney and J. H. White. Preliminary observations of bacteriophage infecting *Xenorhabdus luminescens* (*Enterobacteriaceae*. *Experientia*, 45, 191, 1989.
21. Richardson, W. T., Schmidt and K. H. Neelson. Identification of an anthraquinone pigment and hydroxystilbene antibiotic from *Xenorhabdus luminescens* *Applied Environmental Microbiology*, 54, 1602, 1988.
22. Schmidt, T.M., B. Blekley and K. H. Neelson. Characterization of an intracellular protein from the insect pathogen *Xenorhabdus luminescens*. *Applied Environmental Microbiology*, 54, 2793, 1988.
23. Schmidt, T.M. K. Kopecky and K.H. Neelson. Bioluminescence of the insect pathogen *Xenorhabdus luminescens*. *Applied Environmental Microbiology*., 55, 2607, 1989.
24. Thomas, G. M. and G. O. Poinar, Jr., Amended description of the genus *Xenorhabdus*. *International Journal of Systematic Bacteriology*., 33, 878, 1983.

### INVOLVEMENT OF LUMINOUS BACTERIA IN THE NEMATODE/INSECT LIFE CYCLE

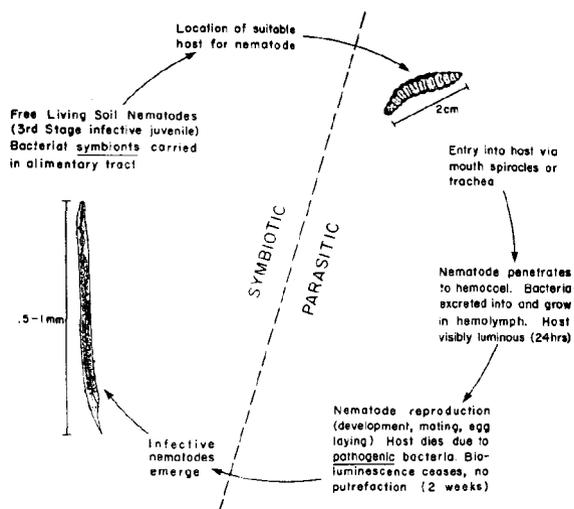


Figure 1.

### ANTHRAQUINONE PIGMENT PRODUCED BY *X. luminescens* (HK)

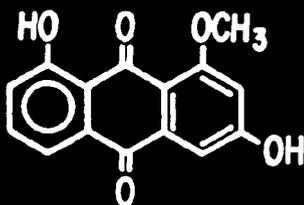
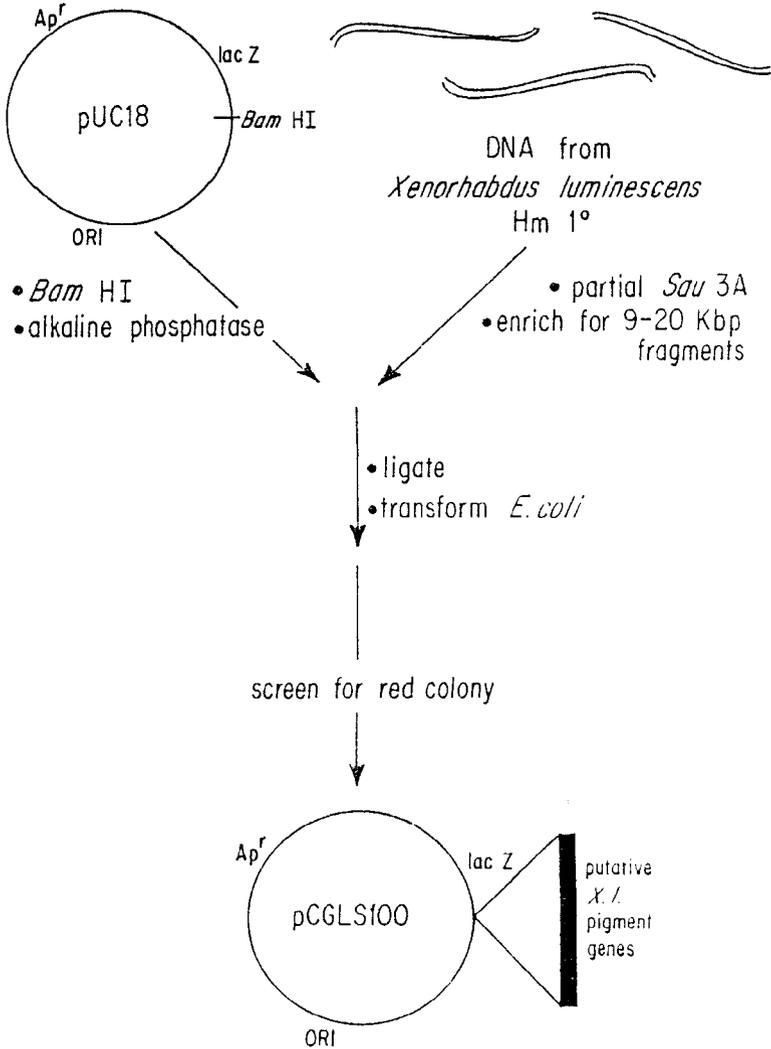


Figure 2.

Figure 3. Cloning strategy.



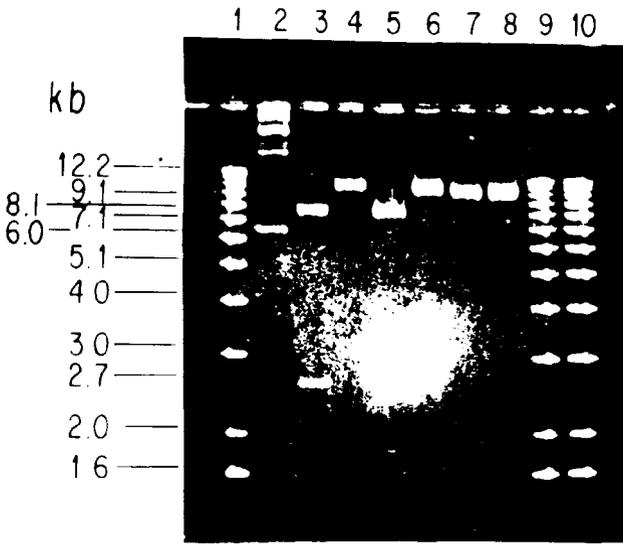


Figure 4. Gel picture of the 8.1 kb insert.

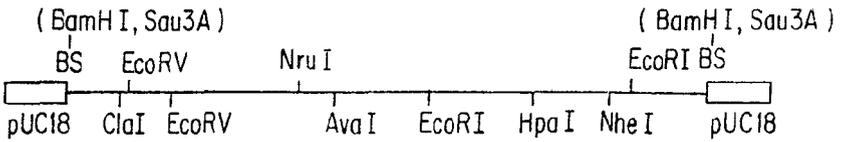


Figure 5a. Restriction map of the insert.

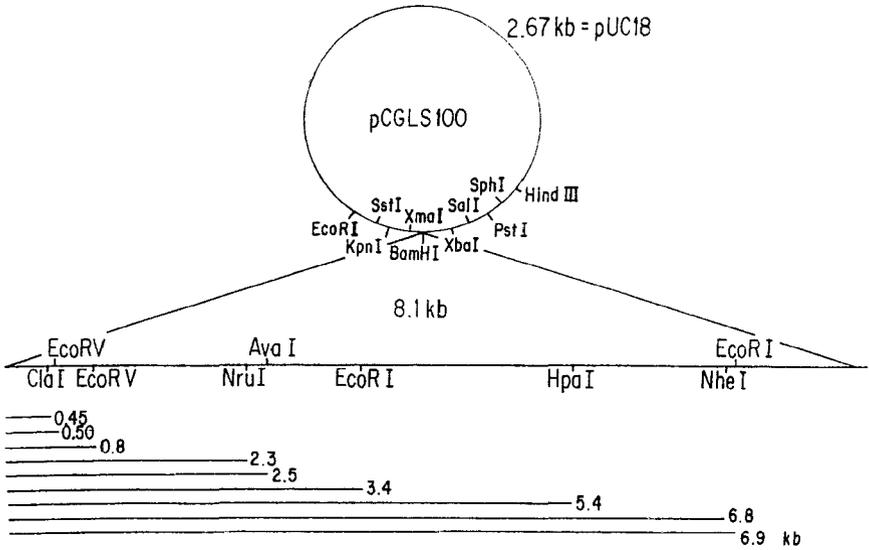


Figure 5b. Restriction map of the recombinant plasmid.

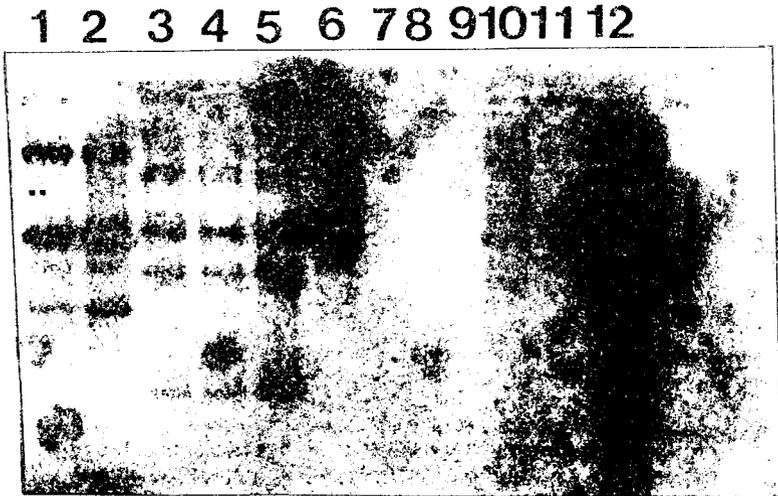


Figure 6. Southern blots of the different *Xenorhabdus* strains with the probe.

**Table 1. Restriction enzymes used in the experiment.**

Restriction enzyme	10X Buffer #	Recognition site	# of sites in the insert
Cla I*	1	ATCGAT TAGCTA	1
Eco RI+	3	GAATTC CTTAAG	2
Eco RV*	2	GATATC CTATAG	1
Ava I+	2	CTCGGG GAGCCC	1
Hpa I*	4	GTTAAC CAATTC	1
Nhe I*	4	GCTAGC CGATCG	1
Nru I*	7	TCGCGA AGCGCT	1

\*Cannot cleave the vector

+ Can cleave the vector

**Table 2. Different *Xenorhabdus* strains.**

Species	Strain	
<i>Xenorhabdus luminescens</i> Hm primary	Hm secondary	
	NC19 primary	
	NC19 secondary	No pigment
		With pigment
	Ela primary	
	27A	
	JW11	
<i>Xenorhabdus nematophilus</i>		
<i>Xenorhabdus poinarii</i>		

# DEVELOPMENT OF A NEW PROCESS FOR COCONUT OIL EXTRACTION

*Chay B. Pham, Merlita T. Punzal, Arlene R. Moreno, Lilia M. Soriano and William G. Padolina*

National Institutes of Biotechnology and Applied Microbiology (BIOTECH),  
University of the Philippines at Los Banos, College, Laguna, 4301 and\*  
University of Sto. Tomas, Metro Manila.

## ABSTRACT

Quantity of coconut oil and copra residue export has been consistently decreasing due to poor quality of coconut oil and copra meal which are contaminated by aflatoxin. This study aimed to develop a new process of obtaining good quality oil using the gamanase-enzyme for the extraction of coconut oil directly from fresh coconut meat.

The effect of different enzymes, substrate concentration, mixing speed, reaction time, enzyme concentration, temperature, and pH was optimized in a batch process. Results show that the yield of coconut oil was 89-90% with gamanase-enzyme at 0.72 ml/100 g of substrate, substrate concentration of 1:3 (w/v), pH 4.5, mixing speed of 300 rpm, temperature of 50°C, for 4 h of reaction time. Coconut oil extracted was colorless and has flavor of fresh coconut meat. High quality of coconut protein and fiber were also obtained. The protein isolated was 8.60%/100 g (d.b) of coconut meat and white. The coconut fiber was white and could be used to produce good quality filter-paper or paper.

## INTRODUCTION

Coconut oil, at present is obtained from dried copra meal by pressing. However, the copra-derived oil has to undergo drastic refining due to mold contamination during storage and deodorizing processes. This removes most of the desired tocopherol substances that inhibit the processes of oxidative rancidity. The occurrence of aflatoxin in copra meal due to improper handling of copra meal by farmers affected the exportation of this commodity. The quantity of coconut oil-export made from copra has been consistently decreasing from 1986-1991 (11) due to the decrease in coconut oil. The improvement of the traditional processes producing coconut oil and meal is necessary to have superior quality products.

Oil extraction technology is considered to date from the invention of the hydraulic press by Bramah in the late 18th century. This process consists of applying hydraulic pressure to oil bearing materials contained in bags, cages or clothes. It is a batch type operation and is labor intensive. Consequently, it has been superseded by continuous pressing using a screw press or expeller or by solvent extraction, or by a combination of these methods (3). All the processes are primarily intended to produce edible oil. As a result, very little, or no atten-

tion is given to the protein residue which is used as animal feed (14).

In the past four decades, many researchers have developed wet processing of coconut oil. Impulse rendering process is described by a series of high speed impulses transmitted through the medium of a liquid, whereby the fat in the cells is liberated and is removed by the liquid in violent movement (4). In 1963, Screenivasan (15) developed the production of emulsion by pressing the fresh kernel. The recovery of coconut oil from emulsion was carried out by the application of heat (12) and freeze-thaw operation (13). The aqueous extraction process developed in the Food-Protein Research and Development Center, Texas A & M, University is designed for the simultaneous recovery of oil and protein concentrates or isolates from fresh coconut utilizing the same basic concept (6).

Recently, an enzymatic technique was developed to effect the oil-extraction of rapeseed, sunflower, seed, palm kernels and others (5). Cell wall-degrading enzymes may be used to extract vegetable oil in an aqueous process by liquefying the structural cell wall components of the oil-containing crop. This concept is already commercialized in connection with olive and rapeseed oil processing (10).

This study aimed to develop the process for the extraction of coconut oil using Gamanase-enzyme and optimization of process conditions in a batch mode-operation.

## MATERIALS AND METHODS

### Materials

Twelve and 13 month-old coconuts (*Cocos nucifera* Linn.) were obtained from a coconut plantation in Los Banos, Laguna, Philippines.

Gamanase 1.5L was obtained from NOVO Industri A/S Bagsvaerd, Denmark. It has an activity of 1,500,000 VHCU/g.

Viscozyme 120L was obtained from NOVO Industri A/S Bagsvaerd, Denmark. It has a declared activity of 120 FBG/ml.

Celluclast 250L (Cellulase) was also donated from NOVO Industri A/S Bagsvaerd, Denmark and has an activity of 2580 CMU/ml.

The chemical reagents such as  $K_2HPO_4$ , glucose citric acid and sodium hydroxide were obtained from Merck are of reagent grade.

### Preparation of Substrate

Coconuts were dehusked and the meat removed from the shell. The paring removed the brown testa with a minimum loss of white meat. The meat was grated through a coconut grater.

Two hundred g of the prepared fresh coconut meat in a container were used in each run of the experiment. Distilled water was added. The mixture was then homogenized for 5 minutes using a blender. This was done to increase the acces-

sibility of the enzyme to the substrate. Prior to the addition of enzyme, the pH of the mixture was measured using the Orion Ionalyzer digital pH meter and was then adjusted to the set pH value using either citric acid or sodium hydroxide solution.

### Experiment Set-Up

A 1.5L stir tank bioreactor with water jacket equipped with motor, thermometer, and thermostat water bath system was used. The temperature of liquid-mixture in a bioreactor was maintained at desired setting temperature using heated water circulated by a thermostat water batch-system through a water jacket. When temperature of liquid-mixture reached a desired temperature, the enzyme was introduced and time of the experiment started.

### Selection of Enzymes

Celluclast, viscozyme and gamanase were used for the selection of enzyme for oil extraction. Process conditions were pH 4.5, temperature 55°C, dilution ratio 1:4 (w/v), mixing speed 200 rpm, enzyme concentration 1 ml/1090 g coconut meat and reaction time, 3h.

### Optimization of Process Conditions

Enzymatic extraction of coconut oil was optimized. The process conditions were temperature (40, 45, 50, 55 and 60°C), pH buffer (3.5, 4, 4.5, 5, 5.5 and 6), mixing speed (100, 200, 300, 400 and 500 rpm), reaction time (0.25, 0.50, 0.75, 1.0, 1.25 and 1.5 ml enzyme/100 g coconut meat).

### Coconut Oil Extraction at Optimized Process Conditions

The optimized process conditions as mentioned in the "Results and Discussion" were applied for the yield of coconut oil. The coconut cream was separated by the decantation method. The washing of coconut cream was repeated twice by distilled water which was decanted afterwards. The pooled liquor was combined with the supernatant.

### Extraction of Protein and Fiber Residue

The supernatant was separated from the residue by centrifugation at 3500 rpm for 20 min. at 4°C. The residue was separated and then washed with distilled water. The washing of residue was repeated twice and the liquors were separated by centrifuging using conditions mentioned above. The pooled liquors were com-

bined and used for the determination of protein.

### **Analytical Methods**

#### **Determination of protein**

The protein content of the solution was determined using Lowry method (8) with Bovine Serum Albumin as standard. To 2 ml of supernatant was added 1 ml of 1 N NaOH. This was then heated at boiling water bath for 5 min., cooled and after which 1 ml of 1N HCl solution was added. The sample solution (0.1 ml) was used for the determination of protein.

#### **Determination of coconut oil**

Coconut oil recovery was determined by heating the cream at 95°C for 20 min. Measurement of the volume of oil by the was obtained by the volumetric method.

Determination of moisture content Moisture content was determined by drying samples in an oven at 105°C for 24 h. The difference of weight before and after drying of sampled was reported as moisture content,

#### **Determination of pH**

The pH value of sample aliquots was determined using a digital pH meter.

#### **Determination of fiber residue**

The fiber residue of coconut was determined by drying in an oven at 80°C overnight.

## **RESULTS AND DISCUSSION**

### **1. Selection of Enzymes**

Prior to optimization of process conditions, it is important to determine which among the cell-wall degrading enzymes are best to be used. Figure 1 shows the ability of cell-wall degrading enzymes of coconut meat at enzyme concentration of 1 ml/100 g substrate, to release the coconut cream. It is also noted that there is a positive linear correlation between the coconut cream and coconut oil. It means that the high value of cream obtained also results in the yield of oil.

Among the enzymes, such as cellulase, vizcozyme, and gamanase used, the gamanase-enzyme gave the highest value of extraction yield (65%) as compared

to vizcozyme (52%) and cellulase (37%) at the process conditions given in Figure 1. The cellulast (cellulase) had the lowest enzyme-ability to degrade cell-wall of coconut meat. It was confirmed with the previous study of Kusakabe et al., (7) that the coconut residue contains about 60% of galactomannan and 1-2% cellulose. Therefore, gamanase is preferred to be used in the hydrolysis of coconut cell-wall.

## 2. Optimization of Coconut Meat Dilution or Substrate

Figure 2 shows the optimization of water added to 100 g coconut meat in the volume of oil released. The maximum value of oil extraction was 300 ml distilled water added to 100 g coconut meat. At high or low substrate concentration, the released coconut oil decreased in the extraction process. It could be due to the mobility of enzyme in the substrate and the concentration of enzyme in the hydrolysis-medium. The maximum yield of extraction was 64% with 1:3 (v/w) of substrate. At low ratio of dilution 1:1 of 1:2 (v/w) of substrate, it is possible that the mixing effect due to high concentration of substrate could cause very high viscosity of medium. Subsequently this decreased the mobility of the enzyme. In contrast, at high dilution of 1:4 or 1:5 (v/w) of substrate, the enzyme concentration in the medium decreased and consequently resulted in low extraction of coconut oil.

## 3. Optimization of Reaction Time

Coconut oil released also depended on the reaction time. Figure 3 shows the volume of oil released which increased as time of hydrolysis increased. The exponential phase of oil released was after 2h of incubation and reached at stationary state after 4h. This hydrolysis time is required for the enzyme to diffuse into the substrate and degrade the cell wall of the coconut which can then release coconut cream. The maximum value of extraction yield was 60% of the total oil in coconut meat.

## 4. Optimization of Temperature

One of the important conditions in the activity of enzyme is temperature. Figure 4 shows the optimum temperature of gamanase enzyme in the mixture of reaction. At optimized 50°C reaction temperature, the percentage of extraction was 74.5% of total oil in coconut meat which was highest among the temperature range of 40 to 60 °C. At high temperature of 60°C, the enzyme activity could be decreased and resulted in the lowering of extraction rate.

## 5. Optimization of Enzyme Concentration

Generally, the amount of oil extraction increased in direct proportion to

the concentration of enzyme added to the hydrolysis medium. Figure 5 shows that at 0.25 ml/100 g of coconut meat, the oil extraction was 54%. The optimized gamanase-concentration used was 0.75 ml/100 g of substrate. At high enzyme concentration (1.0 to 1.5 ml/100 g of substrate), the increase of extracted oil yield is not significant.

## 6. Optimization of pH

Figure 6 shows the optimum pH of the extraction medium for coconut oil. At pH 3.5 and 6.0 of extraction medium, the coconut oil released had the lowest values. The optimized pH-extraction medium was 4.5 with a 88.5% yield of oil extracted. At higher pH (6.0), the activity of gamanase decreased and resulting in an extraction-yield of 67%.

## 7. Optimization of Mixing Speed

The mixing reaction medium was also an important factor for the extraction of coconut oil by enzymatic process. With increased mixing speed from 100-300 rpm (Figure 3), the extraction yield increased. However, when mixing speed increased to 400 or 500 rpm, the extraction yield decreased. It could be due to the heat generated from the stress-force provided by mechanical mixing. The optimized mixing speed for enzymatic extraction of coconut was 300 rpm with coconut oil-yield of 89% of the total oil in coconut meat.

## 8. Coconut Oil Yield at Optimized Process Conditions

Average coconut oil yield by gamanase-enzyme from 4 batches of 200 g of coconut substrate carried out was 89-90% at the optimized process conditions as compared with 100% using petroleum ether. This is higher than the mechanical process (Hagenmaier, 6) which gave a 75% oil yield and enzymatic process (McGlone, 9) without optimized conditions which gave an 80% oil yield.

## 9. Protein and Fiber of Coconut

At the optimized process conditions, the average of protein was 8.60% (d.b.) in the supernatant of coconut medium as compared to 10.5% (d.b.) of protein content in 100 g (d.b.) of coconut meat. The yield of protein released by the effect of process-extraction was 81.90%.

The fiber (residue) of coconut meat obtained from the enzymatic coconut oil process was 13.54% (d.b.) which is higher than that obtained by the AOAC analytical method (1). The difference in yield is 1.85% (d.b.). This could be due to protein and fat which are not completely eliminated by the enzymatic process.

## SUMMARY AND CONCLUSIONS

Optimization of process conditions for the enzymatic extraction of oil, protein and fiber in coconut were studied in a batch process using germanase-enzyme. The effect of different enzymes, substrate concentration, reaction time, agitation speed, pH, temperature and enzyme concentration were optimized. The interaction effects of process conditions such as pH, temperature and enzyme concentration also contributed to the high significant yield of coconut oil. At optimized process conditions (pH 4., temperature, 50°C; agitation speed, 300 rpm; enzyme concentration, 0.75 ml gamanase/ 100 g coconut meat), the yield of coconut oil was 88-90%. The oil has water-like color and fresh coconut flavor.

The application of cell-wall degrading enzyme in the processing of coconut oil also contributed to the release of protein and fiber. The protein recovery accounted for 82.30% of the total protein obtained in fresh coconut meat and the yield of fiber was 13.54% (d.b.). The coconut fiber is white and could be used to produce good quality filter-paper or paper.

## ACKNOWLEDGMENT

The authors wish to acknowledge the Philippine Coconut Authority, Quezon City for the research grant and NOVO Industries A/S, Denmark for the donation of enzymes.

## REFERENCES:

2. Banzon, J. A. and J. R. Velasco. 1992. In "Coconut Production and Utilization". Philippine Coconut Research and Development Foundation, Inc., Metro Manila, Philippines.
3. Cater, M. C., K. C. Rhee, R. D. Hagenmaier and K. F. Mattil. 1974. Development of continuous pressing for oil extraction. Journal of American Oil Chemists Society. 51:137-141.
4. Chayen, I. H. and D. R. Ashworth. 1957. The chayen impulse rendering process. Journal of Applied Chemistry. 3:529-534.
5. Christensen, F. M. 1989. Technology of enzymatic extraction of vegetable oil. Biotechnology and Applied Biochemistry. 11:249- 265.
6. Hagenmaier, R. D., C. M. Cater and K. F. Mattil. 1973. The aqueous process of recovery oil and other products from fresh coconut meat. Journal of Food Science. 38:516-520.

7. Kusakabe, I., A. F. Zamora, S. Kusama, W. L. Fernandez and K. Murakami. 1988. Studies on the Mannanase of Streptomyces. VI. Structure of copra galactomannan and specificity of Streptomyces B-Mannanase to the substrate. Japanese Journal of Tropical Agriculture. 30:264-271.
8. Lowry, O. H., Rosenbrough, N. J., Lewis Farr, A. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biology and Chemistry. 193:265-275.
9. McGlone, O. C., A. L. Canales and J. M. Carter. 1986. Coconut oil extraction by new enzymatic process. Journal of Food Science. 51: 695-697.
10. Olsen, H. S. and F. M. Christensen. 1984. In "Proceedings International Symposium on Industrial Basic Foodstuffs for Food Industry, Paris, October 29-31, 1984. pp. 335-356.
11. Philippine National Statistical Office. 1992. Foreign Trade Statistics of the Philippines, p. 506.
12. Robledana-Luzuriaga, P. 1956. Process for simultaneously extracting oil and protein from oleaginous materials. U. S. Patent, 2762820.
13. Roxas, P. G. 1963. Process of recovering oils from oleaginous, meat of nuts, beans, and seeds. U. S. Patent 3083365.
14. Samson, A. S. 2971. Chemical and technological investigations on coconut products. Journal of Science and Food Agriculture. 22:312-316.
15. Screenivasan, A. 1963. FAO/WHO/UNICEF. PAG. Nutrition Document R/a and add. 5.

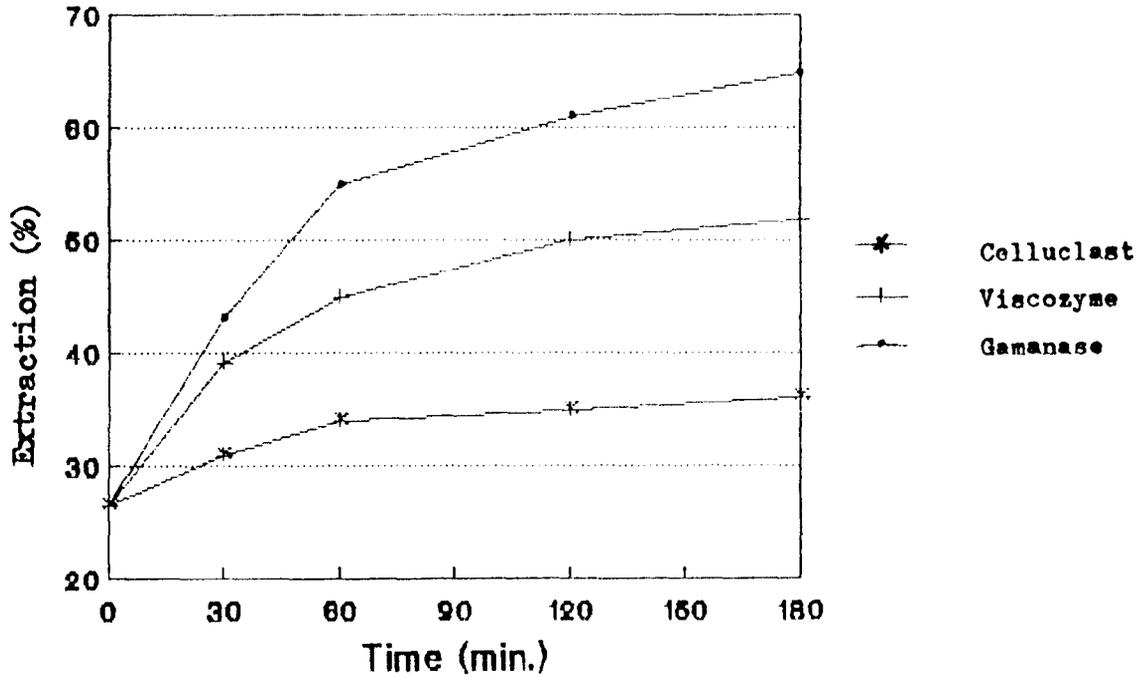


Figure 1. Selection of enzyme for extraction of coconut oil using pH 4.5, temperature 55°C dilution ratio 1:4 (w/v), mixing speed 200 rpm, enzyme concentration 1 ml/100 g coconut meat and reaction time, 3 hr.

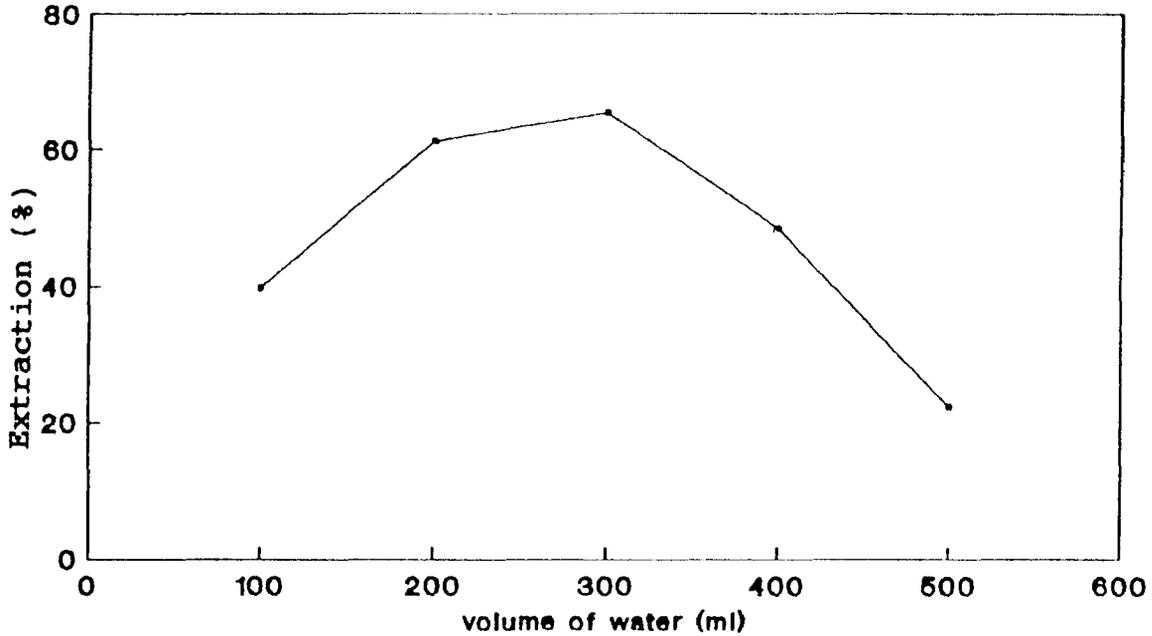


Figure 2. Effect of coconut meat dilution on enzymatic extraction of coconut cream at pH 4.5, temperature 55°C, mixing speed 200 rpm, reaction time 3 hr, and enzyme concentration 1 ml, using 100g coconut meat.

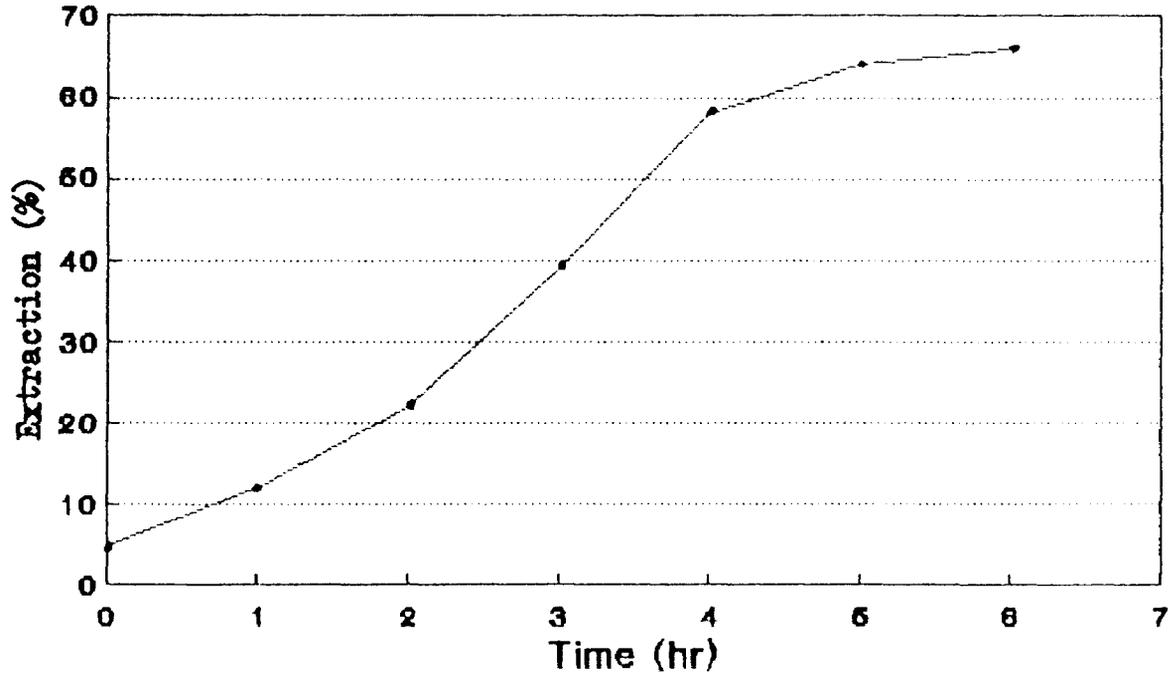


Figure 3. Effect of reaction time on extraction yield of coconut oil using pH 4.5; temperature 55°C; dilution ratio 1:4 (w/v); mixing speed, 200 rpm; and enzyme concentration of 1 ml/100g coconut meat.

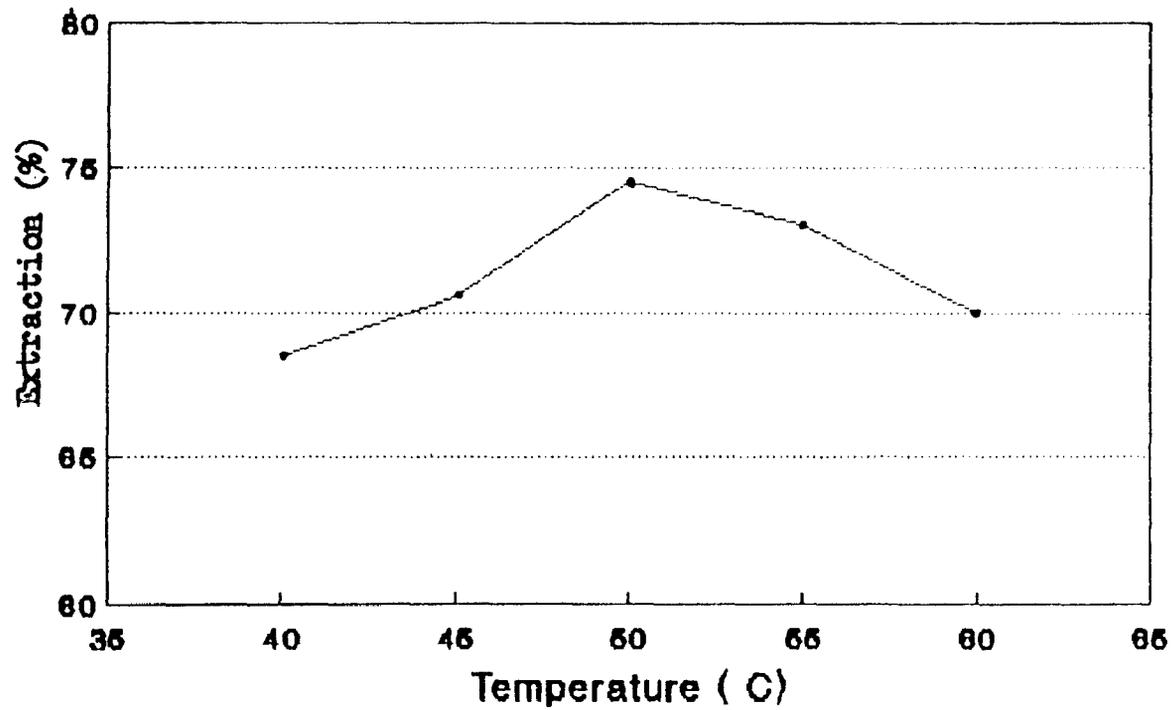


Figure 4. Effect of temperature on activity of enzymes on extraction of coconut cream at pH 4.5, enzyme concentration 1 ml/100 g coconut meat, mixing speed 200 rpm and reaction time 3h.

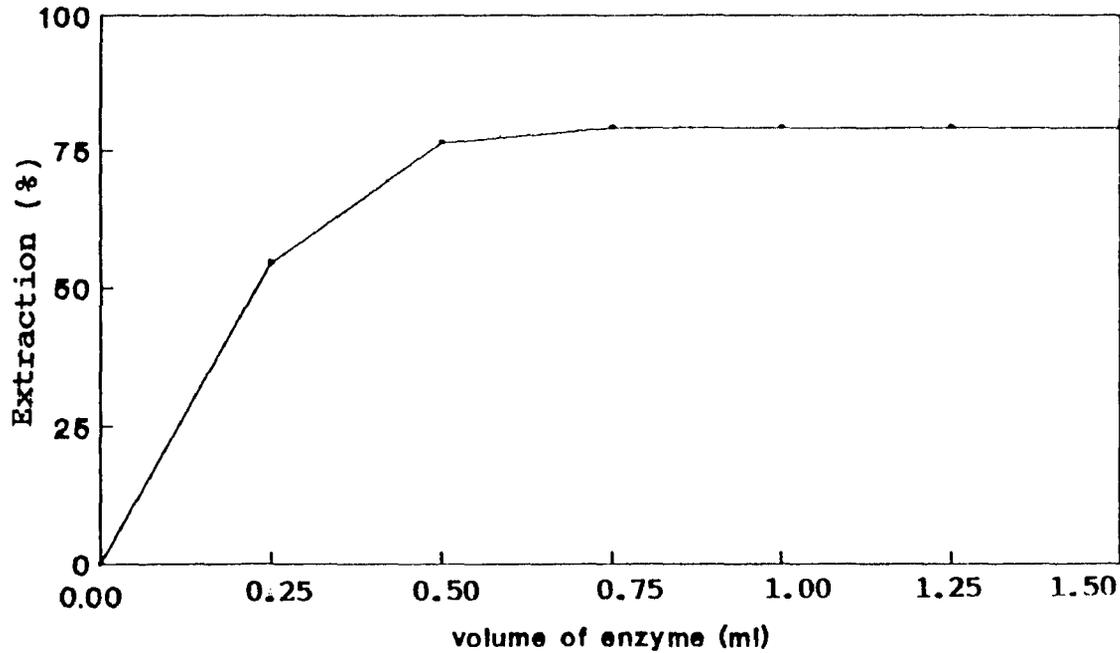


Figure 5. Effect of enzyme concentration on the enzymatic extraction of coconut cream at pH 4.5, temperature 55 C, mixing speed 200 rpm, dillution ratio 1:3 (w/v), and reaction time 3 hr, using 100g coconut meat.

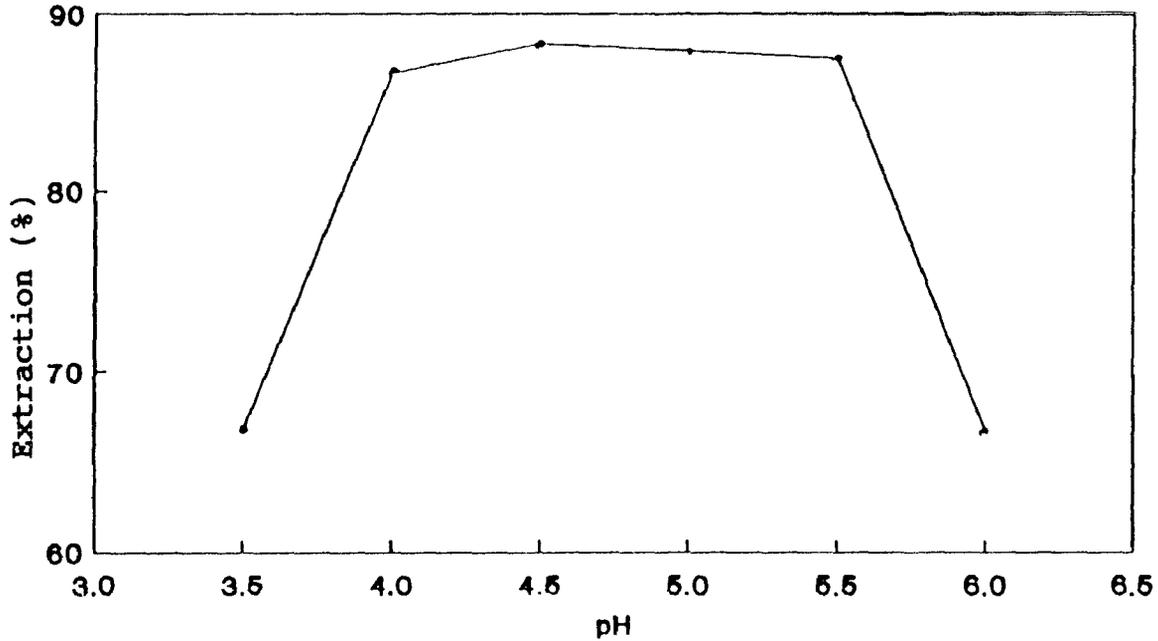


Figure 6. Effect of medium pH on the enzymatic extraction of coconut cream at temperature 50°C, dilution ratio 1:3 (w/v), enzyme concentration 1 ml/100g coconut meat, mixing speed 200 rpm, reaction time 3 hr.

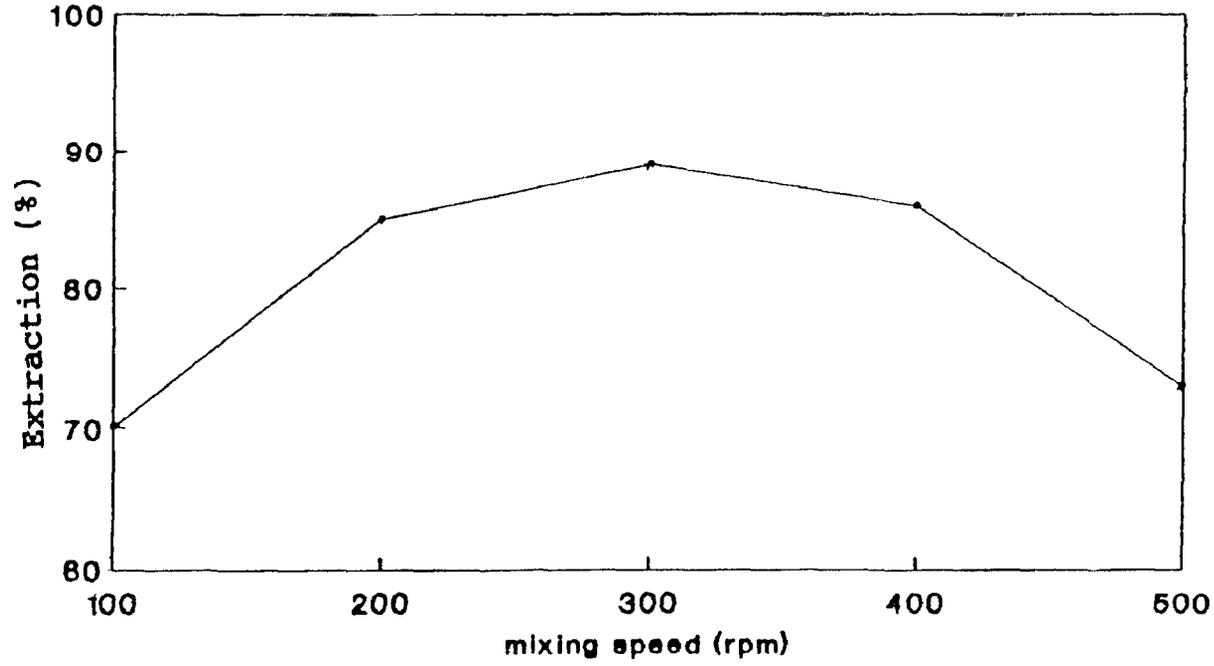


Figure 7. Effect of mixing speed on the enzymatic extraction of coconut cream at pH 4.5, temperature 50°C, enzyme concentration 1 ml/100g coconut meat, dilution ratio 1:3 (w/v), and reaction time 4 hr.



# THE DEVELOPMENT AND OPTIMIZATION OF A COCO-SWEET POTATO CANDY

L. S. Palomar, D.D. Atok and M.E.M. Abit  
Visayas State College of Agriculture  
Baybay, Leyte, Philippines

## ABSTRACT

A coco-sweetpotato candy was developed utilizing fresh cocomilk and sweetpotato. A 3 x 3 factorial with 3 levels each of cocomilk (0-30 g) and sugar (45-75 g) in a candy formulation containing sweetpotato, cocomilk, sugar, starch, peanuts and margarine was used; response surface methodology (RSM) was followed to optimize cocomilk-sugar combination.

Evaluation on cocomilk and sugar level showed that 25 g cocomilk and 55 g granulated sugar per 75 g sweetpotato produced the most acceptable product with either boiled mashed or ground sweetpotato. VSP1 and VSP5 were the most suitable sweetpotato varieties in terms of color and in all the other sensory attributes, respectively. A size of 1 x 7 cm and colored cellophane as wrapper were preferred. All formulations containing at least 45 g sugar with none or even a high level of cocomilk resulted to acceptable products (scores  $\geq 6.5$ ). However, a low level of cocomilk resulted to products with low color and texture scores. Significant but negative correlation existed between texture acceptability scores and % fat and protein.

## INTRODUCTION

Cocomilk is a popular ingredient among Filipino families. However, it is generally used only in native food delicacies ingredient in manufactured foods has already been recognized because of its unique properties. It is also used to increase the fat content of soy milk (Banzon and Escueta, 1968). Attempts have been made to use it as a substitute for canned cow's milk in the processing of cakes and other dessert and snack items (Fementira, 1982).

Sweetpotato (*Ipomoea batatas* L) has the highest sugar and thiamine, and the middle range for energy, protein, starch, dietary fiber and minerals (Bradbury and Holloway, 1988). Furthermore, the availability of different colored varieties make it superior especially in terms of Vitamin A content (Truong et al., 1992).

Candy is a popular product especially among children. Attention should be given then to its nutritional improvement by incorporating other ingredients aside from sugar. Response surface methodology (RSM) has been found suitable to test different variable at a time and optimize formulation. This study was then conducted to utilize fresh cocomilk in the development of a coco-sweetpotato candy, to determine the forms of sweetpotato and type of sugar, to evaluate other ingredients, size and type of wrapper suitable for the product, and to optimize cocomilk and sugar combination using RSM.

## METHODOLOGY

### Extraction of Cocomilk

Mature coconuts were used as source of cocomilk following the extraction method of Banzon and Velasco (1982) with modification. The grated coconut meat was added with measured water kneaded or worked with the hands, placed in a cloth (ca. 30 cm x 30 cm.) and pressed manually with a wringing action to extract the fluid (cocomilk). The resulting press cake was again mixed with water (about 1:1 (v/v)) and repressed. The cocomilk was used immediately after extraction.

### Product Processing

Cocomilk, sugar and starch were combined and cooked at a very low fire until moderate concentrated. All the other ingredients were added and cooking was continued until the mixture thickened. The cooked mixture was transferred to a tray flattened and cut to the desired size and shape. Baking was done for 20 min at 150 C.

### Consumer, Proximate and Statistical Analyses

The processed products were presented to a group of panelist to evaluate their sensory characteristics following standard procedures (Gatchalian, 1989). Proximate analyses were conducted following the procedures adapted by Cagampang and Rodriguez (1980). Statistical analyses followed the procedure of Gomez and Gomez (1980).

### Optimization of Cocomilk and Sugar Combination

**Steps.** The optimization process included the decision on variables. Cocomilk and sugar were used at three levels each. Product processing followed, after which samples from different treatments were subjected to sensory evaluation and consumer testing using the incomplete block design with  $T=9$  and  $K=5$ . The data were used analyzed and regression coefficients determined and used to generate contour plots which were later overlaid to obtain the optimum regions.

**Consumer Acceptance Tests.** Samples were evaluated for acceptability of sensory qualities using a 9-point Hedonic Scale (Peryam and Pilgrim, 1957). Presentation was done using three digit random numbers (Meilgaag, et al., 1987). Forty-five (45) employees of ViSCA in Baybay, Leyte served as consumer panelists. Each panelist evaluated five samples from the seven treatment combinations employing the incomplete block design (Cochran and Cox, 1957). All tests were designed so that each samples was evaluated by at least 24 consumers (SED-IFT, 1981). Samples were presented following the procedure of Mebesa (1986).

**Verification of the Optimized Region.** Verification experiments were conducted in duplicate using three selected treatments. Thirty panelists were randomly selected from the 60 participants of the consumer tests. The treatments included one within the optimum area, one near the optimum, and one outside

these two areas. A T-test was performed to determine if the observed values were different from the predicted values.

## RESULTS AND DISCUSSION

Mashed or ground boiled sweetpotato with white sugar had products with similar scores for almost all sensory attributes except for texture (Table 1). Passing the boiled sweetpotato into a grinder probably rendered it too fine and thus affecting the texture of the finished product. Furthermore, granulated sugar for almost all the sensory qualities regardless of the form of sweetpotato roots since brown sugar has a dark color resulting in a dark and less translucent product. The most popular candies are usually translucent.

There were significant differences among the different sweetpotato varieties; Candies with VSP5 produced the most acceptable product in all sensory qualities evaluated except for color (Table 2). VSP1, an orange variety of sweetpotato, produced a candy with the most acceptable color scores. This may be because candies with the highest sensory scores on general acceptability. VSP5 had medium dry matter content (Truong et al., 1992) which might have contributed to the good aroma, flavor, texture and overall quality of the product.

The other varieties of sweetpotato can be used in the manufacture of this product as shown by the range of scores given by the panelists which were within "like slightly" to "like very much" in the Hedonic scale. This is possible because cocomilk, sugar and cornstarch were also present and the product was cooked to the right consistency and further baked. Thus, the finished product would really be acceptable regardless of the varieties used. For diversity, then, one can produce different naturally colored coco-sweetpotato candy utilizing the different sweetpotato varieties with diverse colors to supply the market with natural, appetizing yet wholesome candy products.

When different levels of cocomilk were used with condensed milk as the control, the candy product with 25 g cocomilk received significantly the highest scores in almost the sensory qualities evaluated (Table 3). This may be because cocomilk has a good flavor which will be more flavorful especially if cooking is extended.

Results also showed that a size of 1 cm x 7 cm was significantly preferred (rank mean 1.90) while 0.5 cm x 12 cm had the lowest rank-level for acceptability (Table 4). For types of wrappers, colored cellophane regardless of candy size was selected (Table 5).

Table 6 shows the mean values for consumer acceptance scores for the attributes tested. Acceptable candies were produced from candies with either the absence of a high level of cocomilk. Mid-level (about 60 g) of sugar gave the most acceptable amount of sugar. It is apparent that a higher amount of sugar has to be used if cocomilk is added in order to attain an acceptable texture. This is logical since this product is a candy with components in addition to sugar which McWilliams (1974) considered as fudge candy.

Proximate analyses showed that as the cocomilk was increased there was a corresponding increase in moisture, fat and protein contents of the products. There was no significant difference, however, on % ash (Table 7). Regression analysis resulted in highly significant ( $p < 0.01$ ) full quadratic regression models for consumer acceptance scores for all attributes except flavor. Full regression equations contributed significantly to the predicted power of the models. Pallomar and co-workers (1994) also observed similar results wherein not all of the terms were used for the flavor regression equation.

Shaded regions represent values for consumer acceptance for a particular sensory attribute corresponding to scores  $\geq 6.5$  (between like slightly and like moderately) as shown in Fig. 1. In general, the optimum regions were leaning towards the sides, lower or higher levels of cocomilk. This indicates that special candy formulation could contain only sugar or other ingredients. Furthermore, the shape of the optimum region for flavor acceptability is almost similar to that of over-all acceptability of the product. The contour plots were overlaid to determine the optimum cocomilk and sugar combination at acceptability scores of  $\geq 6.5$  (Fig. 2).

Texture seemed to be the limiting quality factor. The texture of candies has to be either hard or soft, without or with high amounts of cocomilk. Results of the correlation analysis between consumers' acceptability scores and proximate composition also supported the above observations. Only texture was found to correlate the above observations. Only texture was found to correlate with % fat and % protein. The relationship was also negative which means that at low % fat and % protein, scores for texture were low and vice versa. This is possible since presence of fat makes the food more tender. In this product, the panelists preferred crunchy but hard candy which is only possible at low fat level or soft candy with high amounts of cocomilk. Obviously, the consumers found medium soft texture a negative characteristics for this candy product.

## CONCLUSION

The use of RSM enabled the researchers to test a wide range of levels for cocomilk (0-30 g) and sugar (45-75 g). This work generated nine different cocomilk and sugar combinations vis-à-vis coco-sweetpotato candy formulations for adoption by small processors.

## RECOMMENDATIONS

1. Application of sorbitol or other additives to reduce moisture pick-up and improve shelf-life quality of the product.
2. Optimization of the formulation and processing conditions should be carried out utilizing trained panelists.
3. Pilot testing and subsequent commercialization should be done.

**LITERATURE CITED**

- Banzon, J.A. and J.R. Velasco. 1982. Coconut Production and Utilization. CRDF, Pasig, Metro Manila. 351 pp.
- Bradbury J. H. and W. D. Holloway. 1988. Chemistry of Tropical Root Crops: significance for Nutrition and Agriculture in the Pacific. ACIAR Monograph No.6 Canberra. 201 pp.
- Cagampang , G.B. and F.M. Rodriguez. 1980. Method of Analyses for screening Crops of Appropriate Qualities. 61 pp.
- Claudio , V.S. , S.V. de Leon AND T.T. Aroyo. 1977. Basic Foods for Filipinos. Manila, Philippines. Published by Merriam School and Office Supplies Cor.
- Fementira, G.B. 1982. Substitution of Canned milk with fresh cocomilk in home snack and dessert products. B.S. Thesis, Visca, Baybay, Leyte, Philippines.
- Gatchalian, M.M. 1989. Sensory Evaluation Methods of Quality Assessment and Development. U.P. Diliman, Quezon City, Philippines. 471 pp.
- Gomez, R.A. and A.A. Gomez, 1980. Statistical Procedures for Agricultural Research, with Emphasis on Rice. International Rice Research Institute, Los Banos, Philippines. 294 pp.
- Hagenmaier, R.D. 1983. Dried coconut milk and other new foods from wet process. Coconut Today 1(1) : 36.
- Mabesa, L.B. 1986. Sensory Evaluation of Foods: Principles and Methods. College of Agriculture, U.P. Los Banos, Laguna. 119 pp.
- McWilliams, 1973. Food Fundamentals. New York. London, Sydmet, Toronto. John Wiley and Sons Inc. 501 pp.
- Woodroof, J.G. 1979. Coconuts: Production, Processing Products. 2nd edition. Westport, Connecticut. AVI Publishing Co., Inc. 307 pp.

**Table 1. Mean taste panel scores of coco-S candy using mashed or ground boiled sweetpotato with two different types of sugar<sup>1</sup>.**

Forms + Type of of SP Sugar	Sensory Qualities				
	Color	Aroma	Flavor	Texture	Gen. Accep.
Mashed + white	7.64a	7.75a	7.54	7.46a	7.74a
Mashed + brown	7.25b	7.43b	7.51	7.34b	7.31b
Ground + white	7.65a	7.73a	7.52	7.12c	7.71a
Ground + brown	7.26b	7.41b	7.52	7.16c	7.31b

<sup>1/</sup> Means in column followed by the same letter are not significantly different using DMRT at 5% level of significance.

**Table 2. Mean taste panel scores of coco-S candy with different sweetpotato (SP) varieties<sup>1</sup>.**

SP Varieties	Sensory Qualities				
	Color	Aroma	Flavor	Texture	Gen. Accep.
VSP 1	8.00a	7.50b	7.38c	6.90d	7.09c
2	7.69b	7.23c	7.38c	7.16c	7.45b
3	7.13c	7.25c	7.32c	6.81d	6.93d
4	7.13c	7.69b	7.69b	7.31b	7.54b
5	7.50b	7.75a	7.94a	7.81a	7.81a
6	6.98d	7.50b	7.44c	6.68d	7.12c
7	7.66b	7.56b	7.72b	7.40b	7.82a
Kabite	7.00c	7.38c	7.32c	6.38c	7.63c

<sup>1/</sup> Means in column followed by the same letter are not significantly different using DMRT at 5% level of significance.

**Table 3. Mean taste panel scores of coco-sweetpotato candy with different levels of cocomilk<sup>1</sup>.**

Treatments	Sensory Qualities (ns)					
	Appear- ance(ns)	Aroma	Flavor	Texture	Firm- ness(ns)	General
Control	7.00	7.25b	6.98	6.33b	6.67	7.02b
15 g cocomilk	7.18	6.45c	7.18	6.93a	7.18	7.15b
20 g cocomilk	7.18	7.00b	7.00	6.95a	6.85	7.04b
25 g cocomilk	7.33	7.98a	7.00	7.79a	6.88	7.39a

<sup>1/</sup> Means in column followed by the same letter are not significantly different using DMRT at 5% level of significance.

ns/ - not significant

**Table 4. Mean ranks and rank level for acceptability of candy with different sizes.**

Size (cm <sup>2</sup> )	Mean Rank <sup>†</sup>	Rank Level
3 x 4 (oblong)	2.55	2
5 x 12	4.95	6
1 x 12	3.80	4
1 x 7	1.90	1
1 x 5	4.35	5
2 x 5	3.20	3

<sup>†</sup> Significant at 5% level based on Kramer's Rank Sum Test.  
Rank of 1 = best; rank of 6 = worst

**Table 5. Mean ranks and rank level for overall acceptability of coco-S candy with different sizes and wrappers<sup>1</sup>.**

Types of Wrapping Material	Size (cm) <sup>†</sup>					
	3 x 4	0.5 x 12	1 x 12	1 x 7	1 x 5	2 x 5
Cellophane (colored)	1.20	1.05	1.05	1.20	1.80	1.00
Japanese paper	2.30	2.10	2.25	2.30	2.00	2.30
Cellophane (white)	2.50	2.85	2.85	2.50	2.20	2.70

<sup>1/</sup> Significant at 5% level based on Kramer's Rank Sum Test.  
Rank of 1 = best; rank of 6; worst

**Table 6. Main taste panel acceptability scores of coco-sweetpotato candy with different cocomilk and sugar-combinations<sup>1</sup>.**

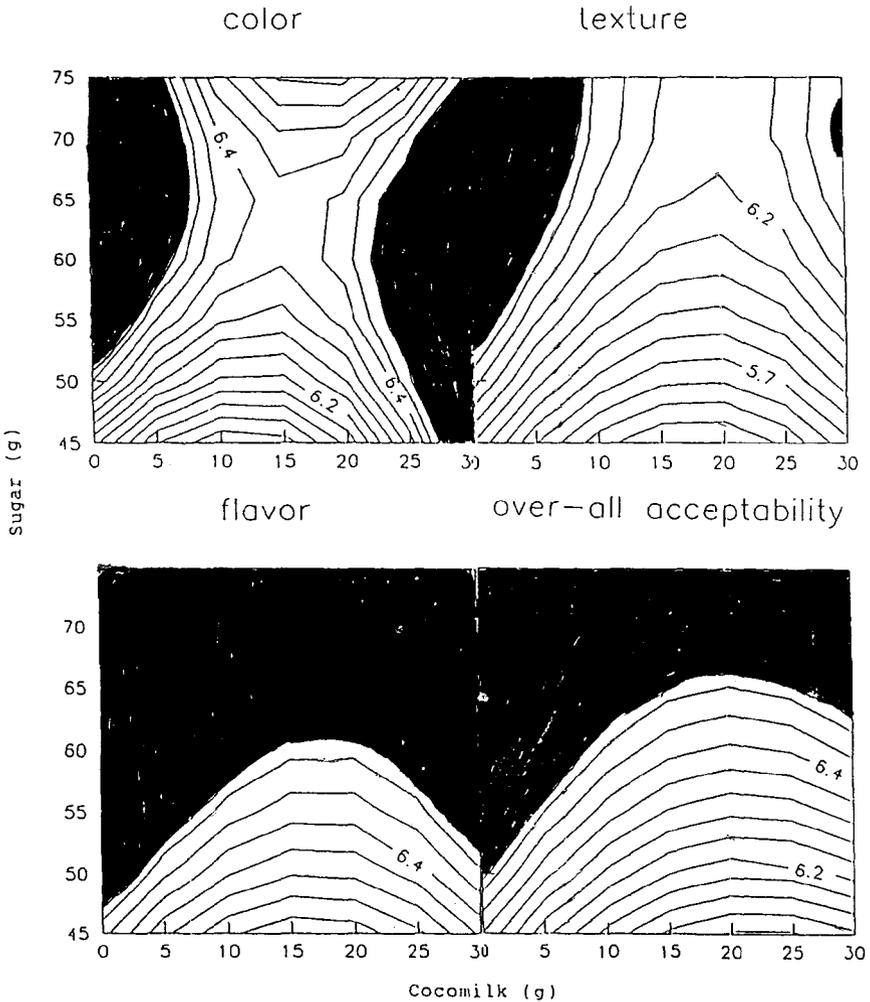
Treatments(g)		Sensory Qualities					Over-all
Cocomilk :	Sugar	Color	Texture	Sweetness	Flavor		
0	45	6.63bc	5.61c	6.52a	6.40abcd	6.62bcd	
0	60	6.83a	6.59a	6.09b	6.86ab	6.86a	
0	75	6.90a	6.88a	5.68bc	6.88a	6.64abc	
15	45	6.12bc	4.96d	5.90bc	6.17d	5.99d	
15	60	6.63abc	5.78bc	6.00b	6.59abcd	6.41abcd	
15	75	6.08c	5.58c	5.85bc	4.43abcd	6.23bcd	
30	45	6.83ab	5.38cd	5.99b	6.38bcd	6.06cd	
30	60	6.53abc	5.63c	5.59c	6.37cd	6.29abcd	
30	75	6.73a	6.28ab	5.60c	6.83abc	6.73ab	

<sup>1/</sup> Means in column followed by the same letter are not significantly different using DMRT at 5% level of significance.

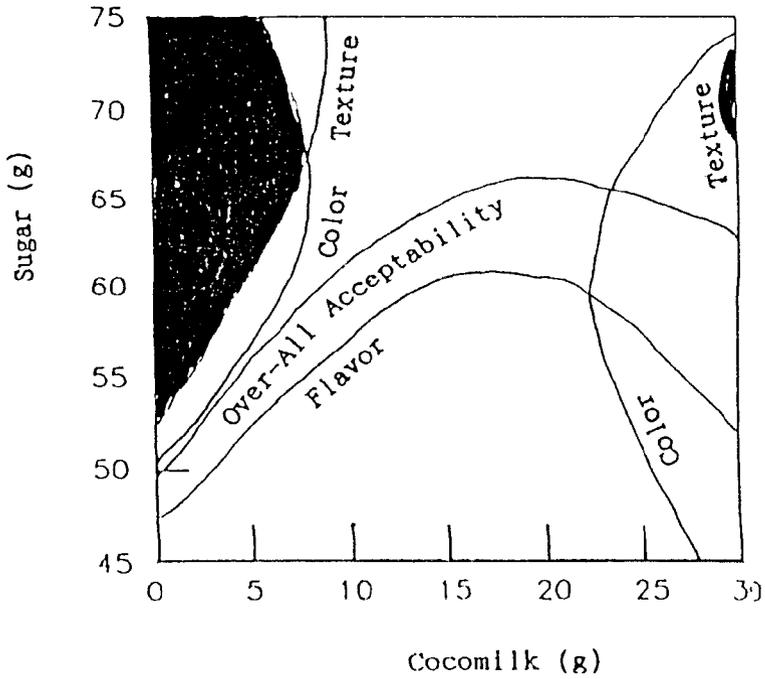
**Table 7. Proximate composition of coco-sweetpotato candy with different cocomilk and sugar combinations<sup>1/</sup>.**

Treatments(g)		Proximate Composition(%)			
Cocomilk :	Sugar	Moisture	Fat	Protein	Ashns/
0	45	4.93d	4.04de	4.04d	0.94
0	60	5.26c	4.94e	4.19d	0.95
0	75	5.28bc	4.10d	4.12d	0.96
15	45	5.39bc	5.48c	4.93c	0.94
15	60	5.38bc	5.49c	5.16c	0.93
15	75	5.58ab	5.48c	5.02c	0.95
30	45	5.83a	6.04ab	6.36ab	0.95
30	60	5.86a	5.99b	6.56a	0.94
30	75	5.89a	6.16a	5.86b	0.91

<sup>1/</sup> Means in column followed by the same letter are not significantly different using DMRT at 5% level of significance.

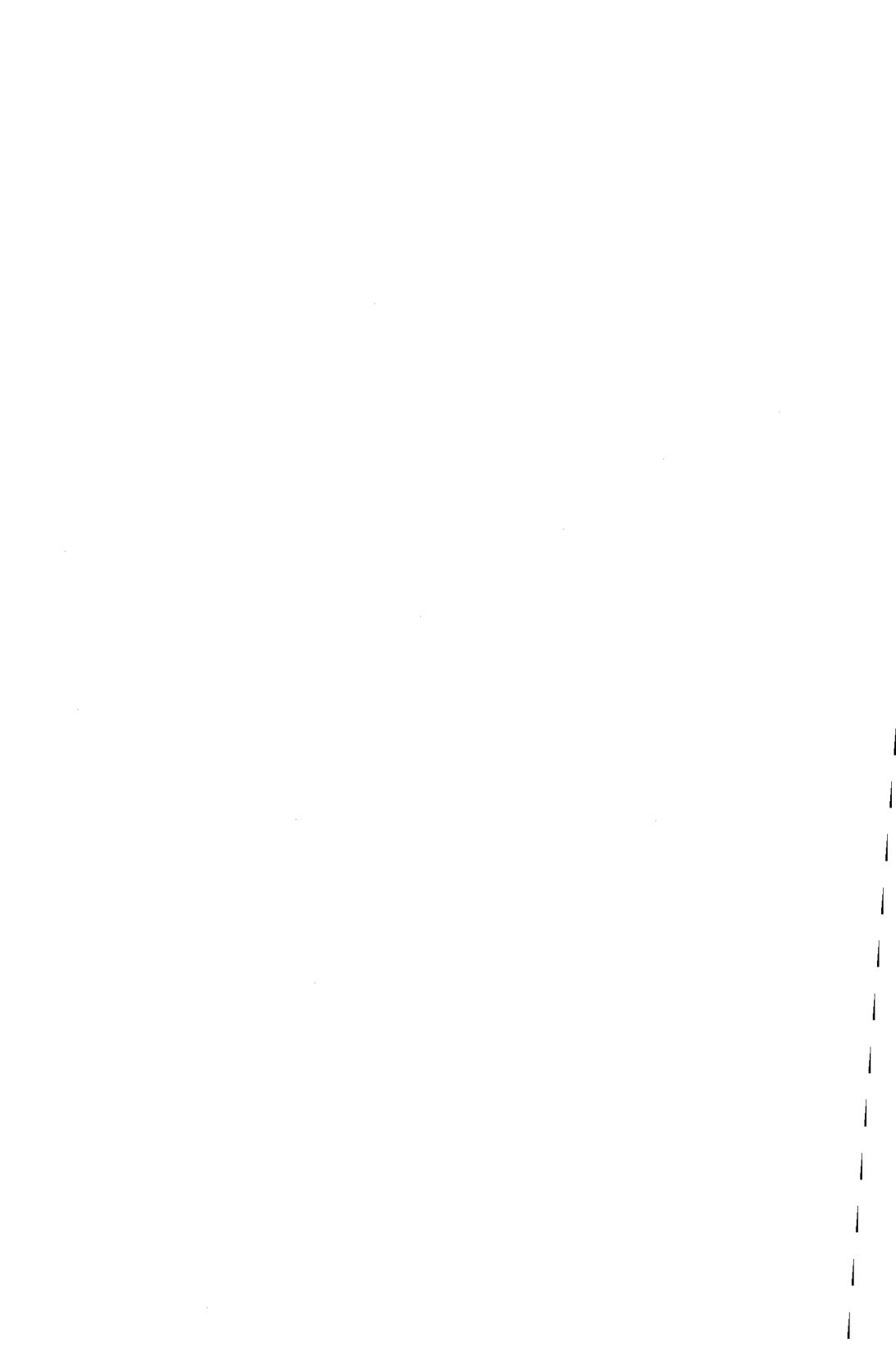


**Figure 1.** Contour plots of sensory properties of coco-sweetpotato candy processed to optimize the formulations. Shaded regions represent acceptance scores for attribute  $\geq 6.5$  using 9-point hedonic scales where 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely.



1

Figure 2. Super imposed regions after overlaying the optimum regions of the different sensory qualities at scores  $\geq 6.5$ .



# FLAVOR ENCAPSULATION IN CARBOHYDRATE MATRICES

*A. Blake, P. Attwood,  
J. Grange, M. Lindstrom,  
Firmenich S.A. Geneva*

## ABSTRACT

The use of carbohydrate glasses as a way of encapsulating and stabilizing flavor systems is increasingly used to provide a very effective method for making products with long shelf life and stability. The glass transition temperature  $T_g$  of such glasses is an important factor controlling their stability and the significance of this is discussed along with the methods of determining  $T_g$ . While this parameter is important it is nevertheless not the only criteria affecting the stability of the products and indeed the diffusion of certain volatile materials through glass carbohydrates appears to be independent of the glass like state. Other factors such as hygroscopicity also play a key role in the stability of encapsulating system and this can have important consequences to product shelf life.

## BACKGROUND

The techniques for encapsulating flavors have been extensively reviewed (4,5,7) in recent years and while spray drying is still a technique much used by the flavor industry there is an increasing use of alternative techniques, many of which specifically exploit the phenomenon of glass encapsulation. I will briefly review this before going on to discuss some further considerations of relevance to this method of encapsulating and protecting flavors and some of the recent work of our company in this area.

## SUGAR GLASS ENCAPSULATION

The idea of making a stable flavored food product by converting crystalline sugar into a clear glassy state through melting it and quickly cooling it, is at least 150 years old and is the basis for much of the confectionery industry. The idea of using the technology to specifically stabilize and encapsulate flavoring systems was not however proposed until 1956 by Schultz et al (6) followed by the initial patent of Swisher in 1957 (8). Since that time a great deal of work has gone into understanding the process, the physical chemistry behind it and the techniques of using it on an industrial scale.

The basic technology behind creating a carbohydrate glass is straight for-

ward (see Figure 1).

Sucrose and most other sugars normally exist in a crystalline state, the most stable form. By melting the crystals their regular structure is destroyed and when this is then rapidly chilled it becomes a clear transparent glass where the molecules of sucrose are frozen in an amorphous noncrystalline form. The melting process is facilitated by addition of water, which reduces the intermolecular forces holding the rigid crystalline structure intact and thus helps the formation of the liquid melt. The water is behaving as a solvent but can also be considered a plasticizer analogous to the role of plasticizers in making hard plastics workable e.g. for extrusion. Mixtures of sugars and other carbohydrates lose their crystalline structure (melt/dissolve) at different temperatures depending on their composition and the proportion of water that is present. When chilled rapidly the molecules do not have time to reorganize themselves into crystals and the greatly increased viscosity of the system locks them into a glassy amorphous state. This glass is metastable and as such will revert to a less energetic, more stable form if molecular freedom becomes sufficient to allow crystals to form; thus increasing the temperature will eventually reach the critical point where the trapped molecules regain sufficient freedom of movement to crystallize, this is usually referred to as the glass transition temperature  $T_g$ . In general when it is in the glassy state the colder the product is relative to  $T_g$ , the more stable and long lived it will be. Exactly the same physical processes control this phenomenon as they do in conventional window glass except in the latter case the glass transition temperature of the silicates in window glass is some hundred or so degrees higher than for sugar glasses and this is the reason why window glass can remain unchanged and without crystallization for centuries.

Given that simple carbohydrate sugars can be converted into a glassy state it is at first surprising that for a long time it was not realized that sugar glasses could provide a very effective encapsulation system for volatile or unstable and easily oxidized chemicals since sugar glasses protect chemicals trapped in them very effectively. The lack of exploitation of this concept was essentially because in order to protect these materials one needs to first get them inside the glass without losing a disproportionate amount of them in the process or causing them to change chemically. It is only in relatively recent years that these problems have been solved practically and on an industrial scale.

Effective encapsulation technologies have evolved and I can mention my own company's range of DURAROME products in this context.

One of the most important advantages of such products is their extreme stability and long life. We have completed strictly controlled comparison tests for such flavors versus conventional spray dried flavors and I show the results of some of these tests in my next slides (Figures 2-4). In this case we have compared the two systems with a selection of citrus oils; both were made using oils from the same batch so that all storage tests started with identical raw materials. During storage the products were regularly screened by a panel of experienced flavorists and objectively by GC of the extracted oils. Although the spray dried flavors have respectably long shelf lives without change it is clear that the

DURAROME encapsulation system is superior.

The long term stability of such systems is also nicely demonstrated by the encapsulation of a relatively easily oxidized pigment; in this case we have used a Paprika oleoresin. Figure 5 shows at the top three bottles containing freshly prepared samples respectively of 7% paprika oleoresin dispersed on salt, 7% paprika oleoresin spray dried on a conventional maltodextrin/modified starch carrier and 7% paprika in a DURAROME matrix. The bottom row of bottles show the same three systems but now after 18 months at ambient temperature where only the last product has retained the paprika colour.

The manipulation of such encapsulation technology and its control is clearly of consideration commercial interest and I would like to discuss some aspect of this.

### THE MONITORING OF GLASS TRANSITIONS

A technology used by ourselves and others for a better understanding of the processes taking place in sugar glasses is that of Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) is a technique used for detecting physical phase changes and for monitoring chemical reactions. Most such processes are endo or exothermic, absorbing or liberating heat energy as a consequence of the change. Very precise monitoring of such energy changes as a function of temperature can reveal considerable detail about these processes and the thermodynamics of them. In practice modern DSC equipment is capable of detecting energy changes as low as 0.2 microwatts. A typical DSC curve which shows a sugar glass/crystal transition is presented in Figure 6 and shows clearly the glass transition temperature and the free energy change associated with the transition. There are interesting differences between a first thermal scan and then a rescan after cooling.

The glass transition temperature ( $T_g$ ) is clearly of some considerable significance because it represents the temperature limit at which the matrix undergoes a relaxation and the onset of molecular movement. It should not be thought, however, that the product undergoes irreversible changes at the  $T_g$  and we are undertaking substantial background research to more precisely understand the significance of  $T_g$ . One of the issues which interests us greatly is the permeability of the encapsulating matrix to small molecules and how this is affected by its temperature and specifically to temperature changes in the region of  $T_g$ . It is clear that carbohydrate glasses are relatively permeable to water in view of the rapidly with which they can equilibrate to different water content in atmospheres of different relative humidity. If water can migrate through a glassy matrix then to what extent can other molecules and how is this affected when the temperature passes through the  $T_g$ ? Other workers (3) have shown that the electrical conductivity of carbohydrate glasses shows no inflection in the region of  $T_g$ , i.e. ions can move as freely through the material when the temperature is above as when below the  $T_g$  (see Figure 7). Le Meste and co-workers have used Electron spin Resonance Studies to look at the mobility of small molecules in polymeric sol-

ids in the region of  $T_g$  and conclude that the diffusion of small molecules in rubbery or glassy material is complex and needs further study<sup>2</sup>. Such observations must bring into doubt some of the now classic ideas about  $T_g$  and its relevance to molecular movement, at least of entrapped molecules. In our own laboratory we have investigated the diffusion of different molecules into glassy matrices using the technique of reverse chromatography. Figure 8 shows the experimental set up where the packing materials in a GC column is the matrix under investigation. A carrier gas of dry helium is passed through the column to a flame ionization detector. Injection of small quantities of the vapor phase of selected organic molecules allows one to see how these molecules interact with the matrix. Figure 9 shows the emergent peaks for a series of chemical species; clearly there is very different behavior and presumably diffusion rates for these different molecules within the matrix.

One thing however is certain, in spite of these observations of different diffusion rates, glassy matrices are notwithstanding extremely effective at stabilizing flavor systems and other factors beside  $T_g$  are probably very relevant to the ultimate stability of such a flavor system.

### THE EFFECT OF WATER ON ENCAPSULATING SYSTEMS

Many carbohydrate systems are hygroscopic and the picking up of moisture can often be deleterious to their storage stability. It is well known that uptake of moisture causes caking of many powdered food ingredients and much classic work has shown that these phenomenon are related to the destabilization of glassy amorphous materials because adsorption of water reduces the  $T_g$  to the point at which crystallization can start<sup>1,9</sup>. Such crystallization can often have an autocatalytic effect since the growing crystals eject water which further depresses the  $T_g$ , causing softening and accelerating the crystallization process. Such massive changes in physical state have dramatic effects on any encapsulated flavors which like water are excluded from the growing crystal mass. It seems likely that the  $T_g$  is only one factor in a chain of events which can lead to instability of encapsulating systems and that the tendency of a system to absorb water from the atmosphere may be an even more important characteristic than a high glass transition temperature in determining the ultimate stability of an encapsulating matrix (Figure 10). Some of our more recent work has concentrated on producing encapsulating matrices which have very much reduced hygroscopicity. What we are finding is that even if two materials have identical values of  $T_g$  then their physical properties and their storage stability is much more a function of their inherent hygroscopicity than any other factor. In this way we are now able to produce encapsulated delivery systems which remain intact when exposed to relative humidities as high as 85% (Figure 11). Clearly such materials will have a part to play in many different applications and under very humid conditions. These will be ideal products for exposure to tropical climates or for use in intermediate moisture food systems which have water contents too low to allow microbio-

logical growth but nevertheless are too humid for existing encapsulation systems to work effectively and for long periods. How we are designing this new range of materials will however have to wait until we are willing to tell the next part of the story.

## REFERENCES

1. Chuy L.E. & Labuza T.P. 1994. *Journal of Food Science* 59 ((No.1), 43-46.
2. Le Meste M/ Voilley A. & Colas B. 1991. Influence of Water on the Mobility of Small Molecules in Water Relationships in Food. Ed. (Levine H. & Slade L) Plenum Press N.Y.
3. Noel T.R. Parker, R. & Ring S.G. March 1994. Paper presented at the Royal Society of Chemistry - Food Chemistry Group (UK) at the Food Macromolecules and Colloids Conference held at Dijon.
4. Reineccius G.A. 1994. Chapter on Flavour Encapsulation p105 in *Edible Coatings and Films to Improve Food Quality*. Technomic.
5. Risch S.J. & Reineccius G.A. 1987. *Flavour Encapsulation ACS Symposium Series* 370.
6. Schultz T.H. et. al 1956. *Food Technology* 10.57 - 60.
7. Shahidi F. & Han X-Q 1993. *Critical Reviews in Food Science & Nutrition* 33 (6) 501-547.
8. Swischer H.E. 1957. US Patent 2,809,895.
9. White G.W., Cakebread S.H. 1966. *Journal of Food Technology* 1.73-82.

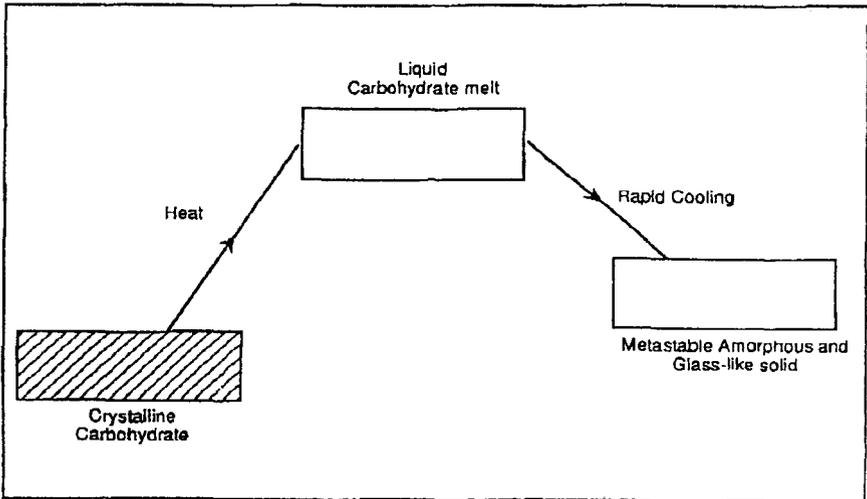


Figure 1. Physical forms induced by heating and cooling carbohydrate systems.

Comparison of Spray Dried versus Glass Encapsulated Flavours after 2 Years Storage at 20°C

	SPRAY DRIED		DURAROME®	
	A	B	A	B
Tangerine	85.7	50	28.5	0
Lime	50.0	33	40.0	0

A - Percentage of tasters who can identify a difference.

B - Percentage of tasters who find the difference unacceptable.

Figure 2.

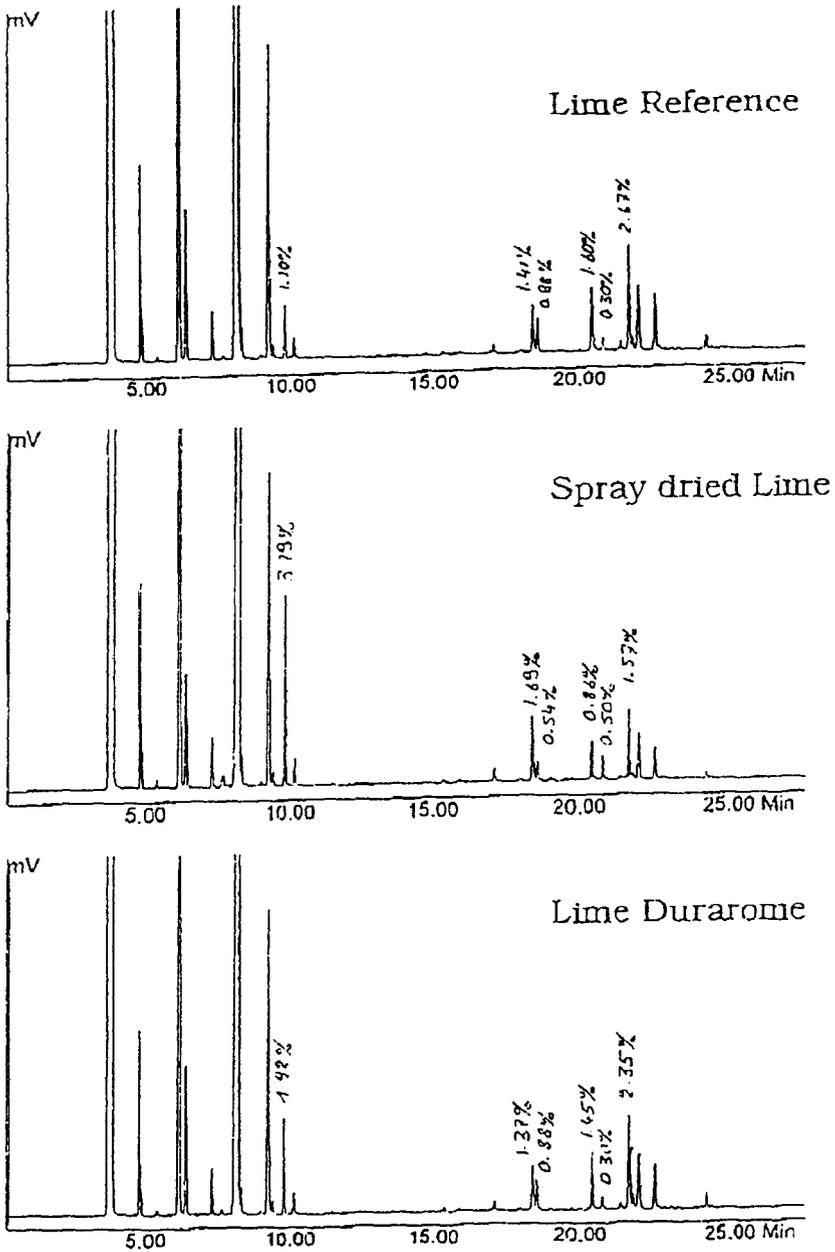


Figure 3.

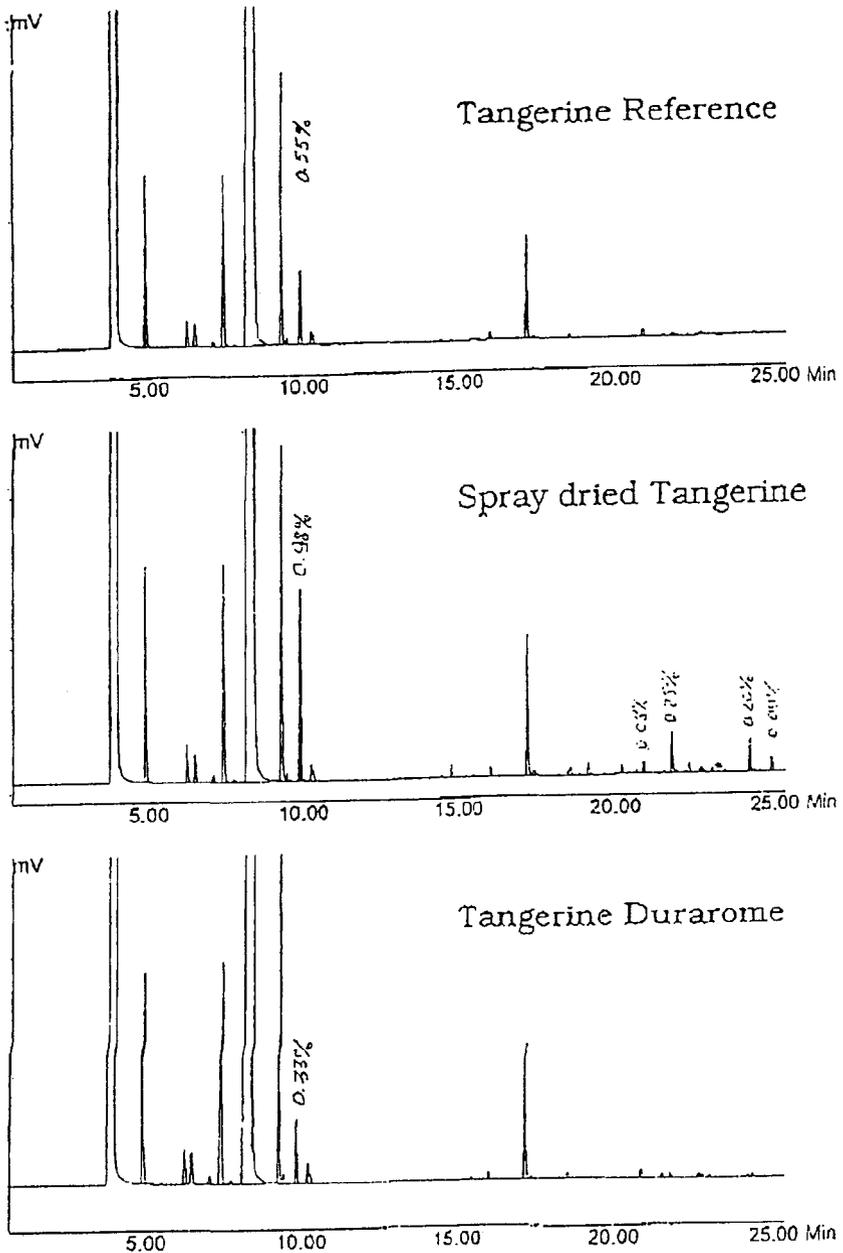


Figure 4.

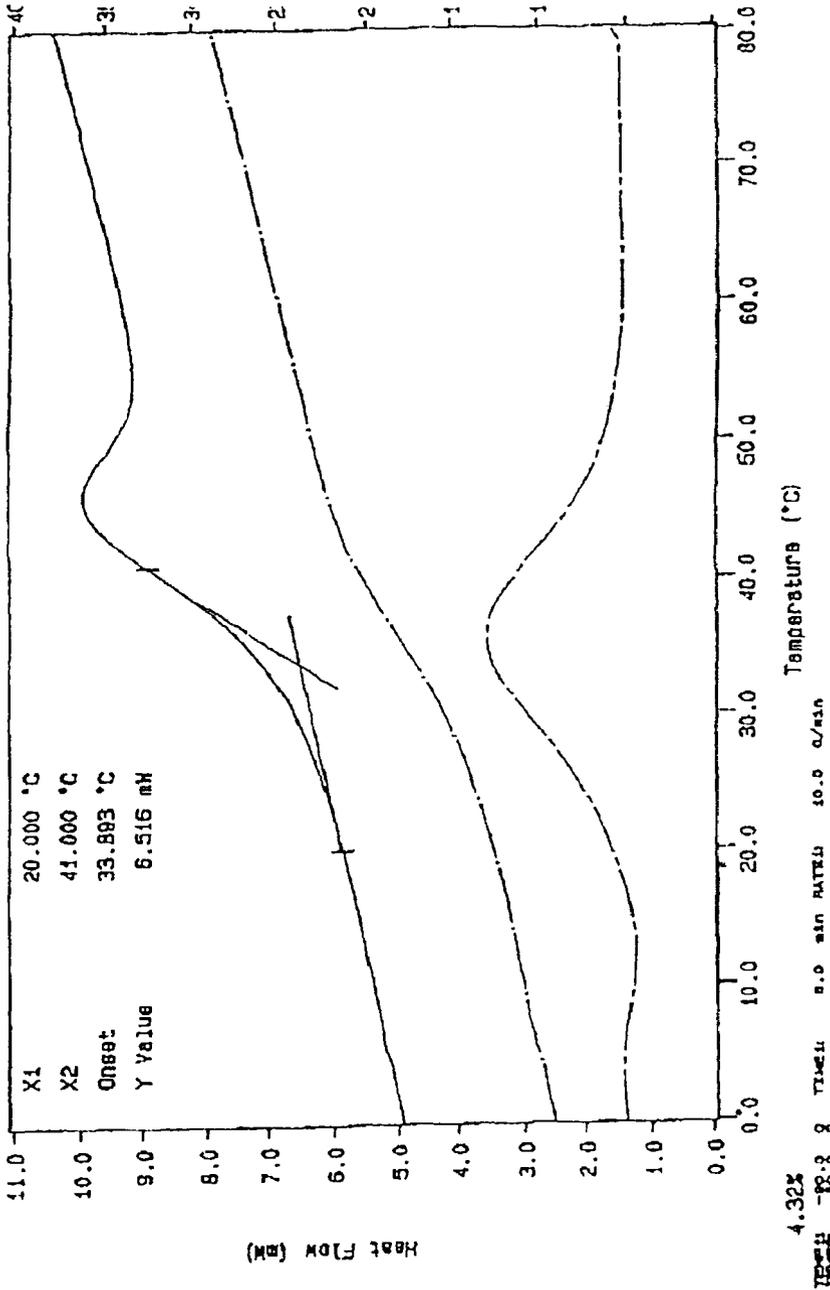


Figure 6.

### Molar conductivity and fluidity of maltose-water-KCl mixtures

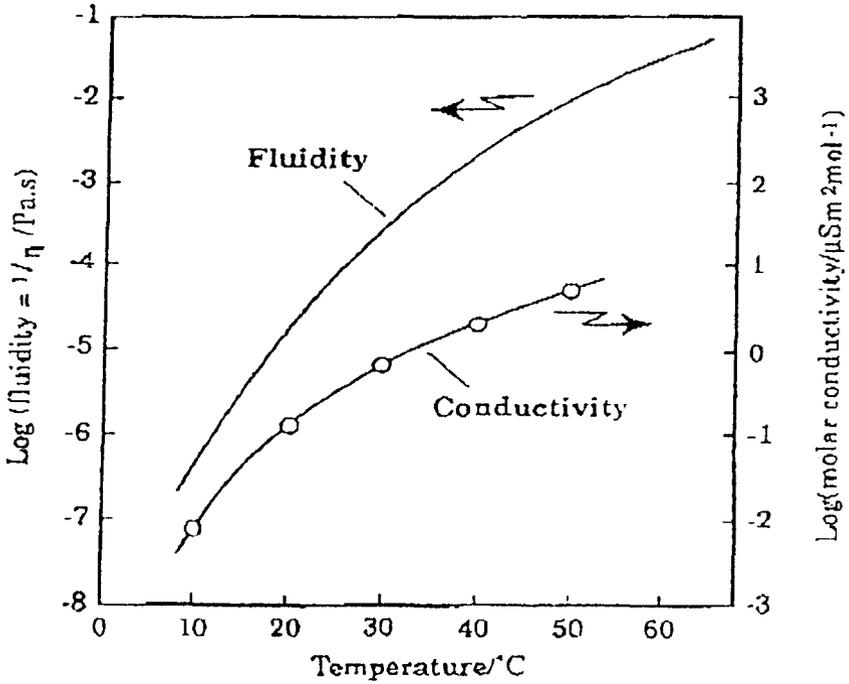


Figure 7. From Noel, Parker & Ring (Ref. 3).

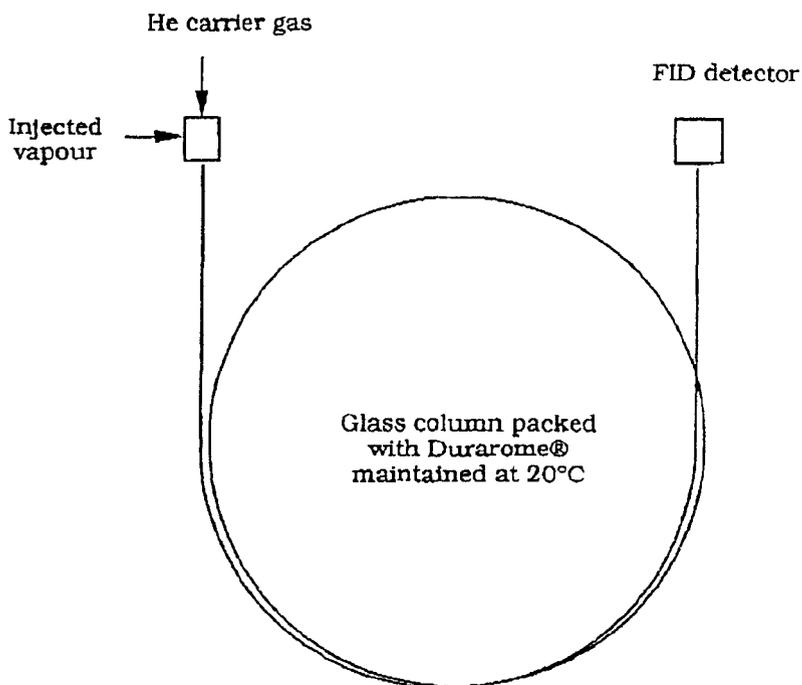


Figure 8. Experimental arrangement for reverse chromatography of Durarome.

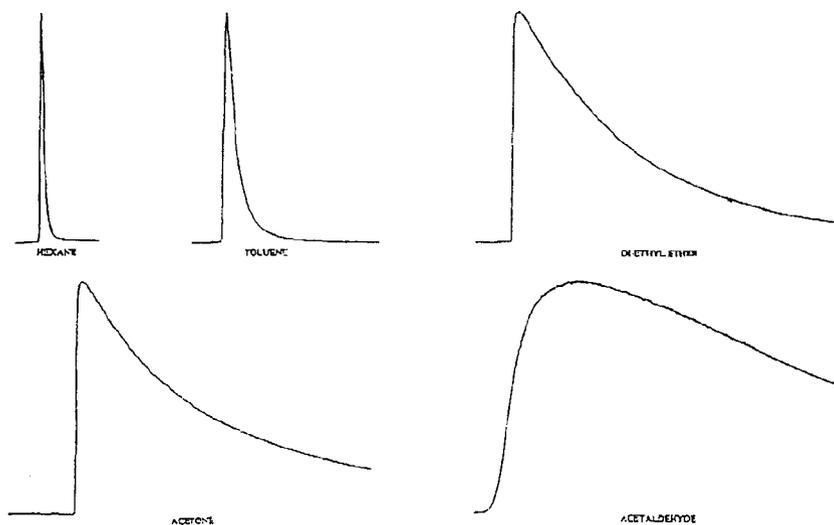


Figure 9.

## HYGROSCOPICITY

leads to

## MOISTURE UPTAKE

causing

## REDUCED $T_g$

giving

## PRODUCT DESTABILISATION

Figure 10.

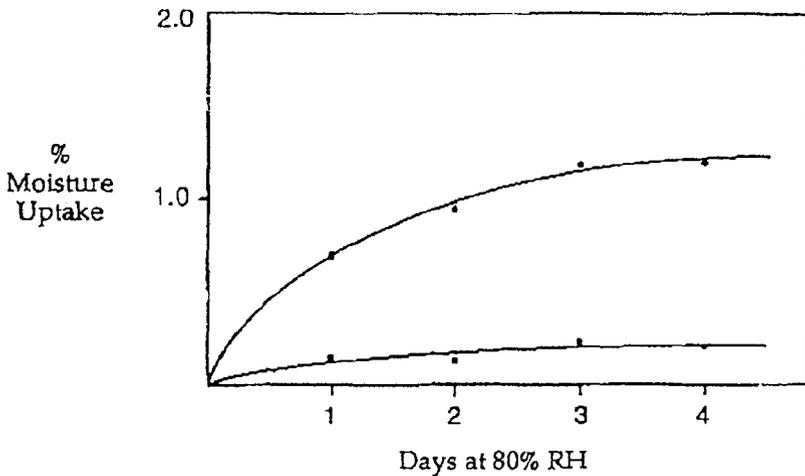


Figure 11. Water uptake of two materials of similar  $T_g$ .

# COMPARATIVE PROFITABILITY OF TWO METHODS OF CUTTING CARCASSES OF CATTLE AND SWINE

*Lourdes S. Rivera, Amorфина G. Galaraga  
Anna Marie N. Austria, and Victoria E. Abella*

Department of Agriculture, Bureau of Animal Industry, Animal Products Development Center, Marulas, Valenzuela, Metro Manila, Philippines 1440

## ABSTRACT

A survey was conducted at 31 Livestock Oksyon Market all over the country and 32 commercial meat markets to assess the practice of cutting hog and beef carcasses. It was found out that cutting procedures varied greatly between markets, although the terminologies used for each cut were the same.

The second part of the study compared the standard USDA cutting procedure with a derived market cut. Using 27 hogs and 10 cattle as samples. Each carcass was cut longitudinally into 2 equal parts. One part was cut following the USDA standard and the other part using the derived market cut. The comparison revealed that cutting hog carcasses by the USDA method increased profitability by 2.6%. For cattle carcasses the profitability advantage was 1.9%.

## INTRODUCTION

Meat are graded to facilitate the marketing and merchandising of meat products. All other purposes or advantages that can be cited are related to some phase of the marketing process, from the initial livestock producer to the ultimate meat consumer.

1. For the livestock producer - grades form part of the basis on which animals are bred, fed, bought and sold. Producers can estimate, with varying accuracy, the grade of animals they plan to produce, the prices they can expect to pay or receive for those animals and which market will buy their production.

2. For the packer, processor and retailer - grading provide a means for segregating animals, carcasses and meat into groups that more uniform as to class, quality and condition, thus producer will supply animals suitable for their particular operation. There is confidence when ordering. By meeting the customers preferences more precisely they can increase their turnover. There will be less waste and more profit as housewives come to accept beef/pork as a reliable, quality product.

3. For the customers, the presence of a grade or brand stamp on a product represents and assurance that the product conforms to some established set of standards, consumers thus have some assurance that the product meets certain standards which coincide with certain standards that they have set for themselves. This allows an easier selection of these products which will most closely satisfy their desires.

Livestock marketing system of the future must continue to be responsive to consumers and manufacturers needs and desires. (Forrest, et. al. 1975)

The standardization of meat carcass cutting throughout the country is the first step towards establishment of quality standards which will benefit both producers and consumers of meat products.

### METHODS AND MATERIALS

A survey was conducted in thirty one (31) Livestock Oksyon Market LOM from Regions I - XII and thirty two (32) public markets (23 in Metro Manila, 6 in Reg. IV and 3 in Reg. III) to determine the practices on grading and marketing of live animals and the marketing practices and cutting procedures of pork and beef carcasses respectively.

For the second, third and fourth part of the research a total of 27 hogs and 10 cattle were purchased from 3 farms in Bulacan, Cavite and Batangas for 3 trials. Three (3) hogs per trial per farm were slaughtered, cut weighed and deboned. The carcasses were cut horizontally into two parts, 1 part was cut according to the market survey (A) cut and the USDA (B) standard cutting method on the other part. The following data were gathered age, sex, breed, weight, dressing percentage, lean-fat-bone ratio, % lean, % fat, % bone, back fat thickness, body and carcass length, % retail cuts and costs. Statistical data was analyzed in a Randomized Complete Block Design.

RCBD Analysis A 3 x 2 factorial experiment in a Randomized Complete Block Design (RCBD) was conducted. The purpose is to determine if there is significant difference between the existing market cut in the Philippines and those of the United States Department of Agriculture in choice retail cut, such as, ham, loin, belly, picnic, and boston butt. Another factor considered in the study is the farm's characteristics that might affect the quality of the hog's carcass like for example farm's hog raising practices, distance and road condition of the farm from the slaughterhouse, etc. There are three trials (considered as blocks) conducted at different times with three experimental hogs each for the three farms namely: CONSOLE FARMS in Bulacan, MONTEREY FARMS in Cavite, and REMNAN FARMS in Batangas. The hogs for each trial were more or less uniform in their physical characteristics such as age, breed and sex.

The experiment hog was cut horizontally into two equal parts but not necessarily equal weights. The market survey cutting method is first applied randomly on one part and then the USDA standard cutting method on the other part. The

measure of analysis used is the percentage of the hog's retail relative to its carcass weight. The factors and their levels are denoted as:

#### **F. Farms under study**

- I. BULACAN
- II. CAVITE
- III. BATANGAS

#### **C: Type of Cutting Method**

HOGS:

- A - MARKET SURVEY CUT
- B - US STANDARD CUT

CATTLE:

- A - US STANDARD CUT
- B - MARKET SURVEY CUT

### **RESULTS AND DISCUSSIONS**

Actual survey of 31 Livestock Oksyon Market (LOM) from Regions I - XII was conducted to determine the practices on grading and marketing of live animals. Survey showed that livestock are brought to the market for sale to the livestock buyers. However, some of the buyers in turn sell it to other dealers in the markets. Livestock are sometimes sold several times, thus prices from various traders add up, resulting to high selling prices to the detriment of the buyers and end users. Few LOM used weighing scale for grading animals while majority of LOM surveyed used subjective grading.

Actual survey on the marketing practices and cutting procedures for pork and beef carcasses were conducted in 32 wet markets (23 in Metro Manila, 6 in Reg. IV and 3 in Reg. III). Results showed that each market have different cutting procedures for pork and beef but the terminologies used for each cut were almost the same. For pork carcass, the shoulder (paypay/kasim) is separated between the 7th and 8th ribs which is different from the standard cuts of 2nd and 3rd ribs while pigue is cut 3 inches away from the aitch bone which is bigger cut than a standard cut. For beef carcass, the shoulder (paypay/pecho) is separated between the 2nd and 3rd ribs and the hindquarter (pierna) is cut between the 9th and 10th as shown in Table I & II.

Because of travel time and short distances of transporting animals from farm to slaughterhouse, quality of meat in terms of color, tenderness, pH, keeping quality, yield in production of meat products are not affected. The Dressing % is within the normal range of 70-80% (Table III). Hogs were not stressed during transport and the animals were rested before slaughter.

The effects of factor F, the farm under study, are insignificant as well as the effect of interaction between factors F and C. This implies that if a hog from BULACAN FARMS and another hog from BATANGAS FARMS are both chopped

either through market survey cut or USDA standard cut, the percentage of the retail cuts is more or less similar.

Table III also shows the prevailing price per kilogram as of first week of July 1994 of the retail cuts at Metro Manila as well as the total revenue resulting from the two cutting methods. A carcass weighing 58.16 kilograms (head-off) will result to 57 kilograms when chopped into retail cuts. The carcass chopped into market cuts will have a total revenue of P4,924.60 while the carcass chopped into standard cut will have a total revenue of P4,938.50 excluding revenues from the sale of head, blood, and internal organs.

If the cost of acquiring a hog is P55.00 per kilogram live weight, a Large White male hog weighing 80 kilograms will have a total cost of P4,400. The gross income derived using the market cut is P524.60 while the gross income derived using the USDA standard cut is P538.50.

Thus, results of the study showed that cutting of hog's carcass using USDA standard cut is P14.00 (2.6%) more profitable than survey market cut.

The cattle were slaughtered at Food Terminal Incorporated (FTI) and carcasses were chopped using two cutting methods. The average carcass weight of the three (3) cattle was 189.4 kilograms and the average dressing percentage was 58.82 percent. Data shows that the lean meat of the oldest cattle at 7 years is 62 percent which is of 60.60 percent and 54.20 percent respectively. The carcass length of all the three (3) cattle are more or less the same with an average of 60.5 inches. The oldest cattle has a carcass grade of class 3, while the other two cattle are of class 2. All the cattle have reddish brown color of lean meat.

Table IV shows that retail cut percentage obtained using the market cutting and standard cutting methods show significant differences. The chuck is the biggest portion in both cutting methods, while the foreshank is the smallest portion except for cattle 3 in market cut where the flank is the smallest. The rump, sirloin and flank retail cuts are consistently larger in the market cut for all the three (3) cattle.

The cost of the live weight of cattle is P60.00 per kilo and 332 kilogram of cattle is worth P19,920. The beef carcass chopped into market cuts have a total revenue of P25,407 while US standard but have P25,908 which is P501.00 (1.9%) more profitable than market cuts, excluding revenues from sale of head, tenderloin and internal organs.

## CONCLUSION

Livestock Oksyon Market (LOM) established by the government allow proper livestock handling, grading and good price to producers. The USDA standard cutting procedures for Pork and Beef showed a difference of P14.00 (2.6%) and P501.00 (1.9%) than market cuts respectively.

The developed grade standards for cattle and hogs and their carcasses will be introduced to the different sectors of the meat industry for each applicability. Thus, seminars will be conducted for this purpose.

**REFERENCES:**

- o John Thomas PCV, Instruction for Proper Grading of Live Cattle and Swine. *BAI Recorder*, Oct. - Nov. 1975: 15
- o Forrest et. al. (1975) Freeman & Company. Meat Grading and Standardization. *Principles of Meat Science* 15:331-353

**Table 8. Characteristics and measurements of cattle at farm in Cavite.**

	Cattle 1		Cattle 2		Cattle 3		Average	
Age	7 years		4 years		4 years		5 years	
Breed	Local Brahman		Local Brahman		Local Jersey		--	
Sex	Female		Male		Male		--	
Liveweight	282.0 kgs		332.00 kgs		352.00 kgs		322.00 kgs	
Carcass weight	160.0 kgs		194.00 kgs		214.20 kgs		189.40 kgs	
Dressing percentage	56.74%		58.43		60.85		58.82	
Lean	62.00%		60.60		54.20%		58.90%	
Fat	11.80%		17.90%		14.00%		14.60%	
Bone	15.80%		14.90%		16.10%		15.60%	
Lean: Fat Bone ratio	5:2:1.0:1.3		3.4:1.4:0.8		3.9:1.0:1.2		4.0:1.0:1.1	
Carcass length	60.00 inches		61.50 inches		60.00 inches		60.50 inches	
Carcass Grade	Class 0		Class 2		Class 2		--	
Color, Structure of lean	reddish brown		reddish brown		reddish brown		--	
Retail Cuts Percentage	A (%)	B (%)	A (%)	B (%)	A (%)	B (%)	A (%)	B (%)
Round	5.20	9.40	5.40	8.00	8.30	4.30	6.30	7.23
Rump	3.10	5.00	4.80	7.70	2.80	4.50	3.57	5.73
Sirloin	5.20	5.70	3.50	4.90	3.80	4.10	4.17	4.90
Shortloin	3.80	3.60	2.50	5.80	3.80	4.10	3.37	4.50
Rib	6.30	5.90	6.20	7.70	5.20	7.60	5.90	7.07
Chuck	11.60	11.20	13.80	11.30	11.60	10.30	12.33	10.93
Flank	1.80	3.50	1.60	3.80	1.10	3.50	1.50	3.60
Plate	3.10	-	3.20	-	3.50	-	3.27	-
Brisket	2.10	3.50	3.20	2.70	3.60	3.80	2.97	3.33
Foreshank	1.50	1.70	1.30	1.50	1.40	1.40	1.40	1.53
Hindshank	2.20	2.00	1.90	1.70	1.90	1.90	2.00	1.87
	-----	-----	-----	-----	-----	-----	-----	-----
	45.90	51.50	47.40	55.10	47.00	45.50	46.77	50.70

A - US Standard cut    B - Survey (Market cut)

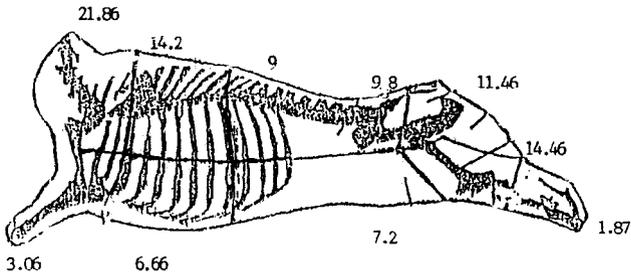
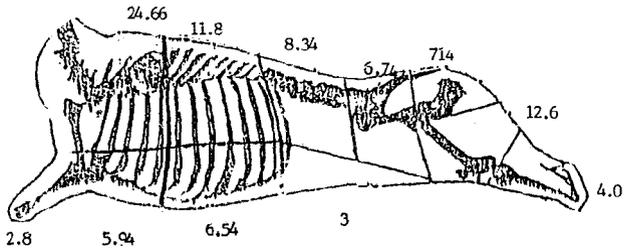
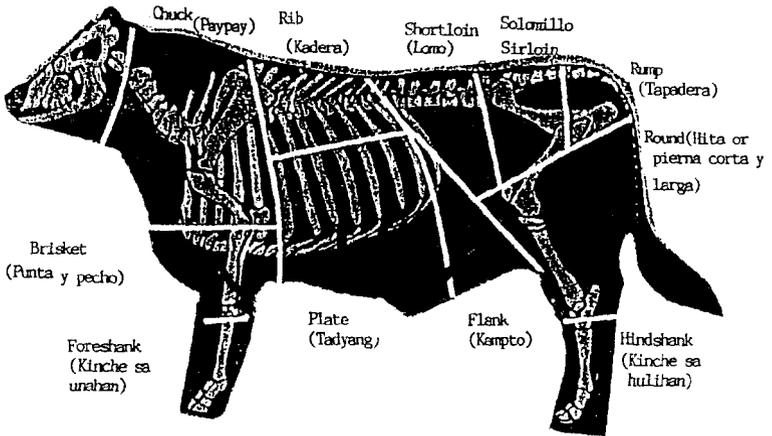
**Beef**

Liveweight = 332 kgs  
 Price Liveweight = P 60.00  
 Total Cost = P 19,920

RETAIL CUTS		A Standard		B Market Cut	
	Price/kg	kg	P	kg	P
Round	P 150	10.55	1,582.50	15.52	2,328
Rump	150	9.3	1,395	9.51	1,426.50
Sirloin	150	6.75	1,012.50	11.22	1,680
Shortloin	150	4.9	735	7.3	1,022
Flank	140	3.1	434	3.41	341
Hindshank	100	3.6	360		
Chuck	140	26.75	3,745	21.91	3,067.40
Ribs	140	11.95	1,673	14.93	2,090.20
Brisket	140	6.25	875	3.31	463.40
Plate	140	6.3	882		
Foreshank	100	2.6	260	2.85	285
		92 kgs	P 12, 954	90 kgs	P 12, 703.50

A P 12, 954  
 B 12, 703.50

-----  
 Half Carcass P 250.50 (1.9%)  
 Whole Carcass P 500.00



**PORK**

Liveweight = 80 kgs  
 Price Liveweight = P 55.00/kg x 80  
 Total Cost = P 4,400

RETAIL CUTS	A			B	
	price/kg	kg/wt		kg/wt	
Pigue (Ham)	P 90.00	19.761	P 1,779.30	15.91	P 1,431.90
Loin	88.00	6.291	553.52	12.29	1,081.52
Belly	88.00	6.66	586.00	10.36	911.68
Boston butt (paypay)	90.00	10.71	963.90	7.21	648.90
Picnic (kasim)	90.00	9.07	816.30	7.55	679.50
others (fore feet) (hind feet)	50.00	4.51	225.50	3.70	185.00
TOTAL REVENUE			P 4,924.60	P 4,938.50	
COST			4,400.00	4,400.00	
GROSS INCOME			P 524.60	P 538.50	

whole carcass

A. Market	P 524.60	
B. Standard	538.60	
	-----	2.6%
Difference	P 14.00	

HOGS

	FARM I	II	III
Age	6 mos.	6 mos.	6 mos.
Sex	male	male	male
Breed	Large white	Large white	Large white
Distance of farm to slaughterhouse	40 kms	65 kms	82 kms
Color and structure of lean	pinkish red	pinkish red	pinkish red
pH	6.6	6.7	6.5
Liveweight	79.32 kgs.	81.09 kgs.	76.40 kgs.
Carcass Weight	59.16 kgs.	58.14 kgs.	54.88 kgs.
Dressing %	74.44%	71.45%	71.41%
Lean Fat Bone ratio	5.2:2.9:1	3.5:2.1:1	3.5:1.75:1
Backfat thickness (inches)	1.3	1.1	1.2
Belly thickness (inches)	1.4	1.2	1.3
% OF RETAIL CUTS	FARM	A MARKET CUTS	B STANDARD CUTS
Pigue (Ham)	I	32.72%	28.65%
	II	35.69	27.78
	III	35.95	27.91
Lomo (Loin)	I	11.12	22.21
	II	11.0	21.0
	III	10	20.05
Liempo (Belly)	I	13.09	18.99
	II	10.85	19.35
	III	11.43	15.76
Kasim (Picnic)	I	14.13	13.5
	II	15.85	11.9
	III	17.89	15.75
Paypay (Boston Butt)	I	19.32	12.41
	II	20	12.14
	III	19.66	14.21

# POST-HARVEST HANDLING AND PACKAGING OF THE THREE (3) VARIETIES OF ANNATTO

*Adoracion A. Ceniza and Virginia TD. Pacaba*  
*Bureau of Plant Industry, Department of Agriculture*  
*Manila, Philippines*

## ABSTRACT

The index of maturity of the three (3) varieties of annatto was established by characterizing and evaluating the carotenoid bixin of the annatto seeds. The results showed that the Pink Flower variety reached its maximum bixin content on the 50th day of maturity. On the 55th day of maturity period, the bixin content of the White Flower and the Native varieties of annatto was fully developed. Characterization of the purified bixin gave a maximum absorption at 470 nm. Analysis of the spectra revealed that the functional groups identified conformed with the functional groups present in the chemical formula of bixin.

Comparison on the quality of commercial annatto showed that the bixin content was below the standard requirement for bixin.

It took 16 hours exposure of the seeds in solar radiation to reduce the moisture content to a level acceptable for safe storage thus attaining the maximum bixin content. The Annatto seeds produced were stable at ambient (28-32°C) temperature for four months storage. They were unstable when held at an elevated (37°C) temperature. The chemical content of the seeds held in both temperatures did not differ significantly.

The polyethylene bags used in packaging and storage add substantial protection from water vapor uptake. It was best for transporting the seeds in bulk because of its adequate strength.

## INTRODUCTION

Annatto (*Bixa orellana L.*) locally known as “achuete” belongs to the Family Bixaceae. It is a large shrub 2 to 5 meters high and grows abundantly in most tropical countries like Bolivia, Brazil, Ceylon, Peru, Dominican Republic, India, Jamaica and Ecuador and to a lesser scale in Kenya, Philippines and Hawaii. The fruits consist of burr-like pods containing numerous seeds. The annatto colors are obtained by extracting the pigments from the pericarp of the fruits. The major pigment present in the pericarp extraction is the carotenoid bixin. This can be yellow, orange, red or violet depending on the concentration of the bixin. Annatto is used mainly in the food industry. Substantial quantity of these are used in the manufacture of cheese and butter, dressings, snacks and confectionery industry. It is likewise utilized in the preparation of various wax polish and as a dye for fabrics.

The use of coal tar dyes in foodstuffs is restricted in the United States. The annatto, a natural dye, is now recommended as an alternative source of color by the Food and Drug Administration of the United States. The same agency, ap-

proved the use of annatto as a colorant whenever yellow to orange-reddish hues are needed in the coloring of food. Since then, the demand of annatto appear to be increasing in the world market.

Annatto is mainly traded in the form of seeds. The main commercial producers and the world's major annatto exporters are Peru, Kenya, Jamaica, the Dominican Republic, Ecuador and Colombia with a high quality seeds. The possible acceptance in the market by other relatively small, non-traditional suppliers will depend on the quality of the seeds they offer particularly on the percentage content of bixin. The importing countries require bixin content of 2.7% or more and the market price of the seeds depend highly on this particular component. With this standard requirement for annatto, it seems that there is a need to improve our technology for the production of high quality annatto. We would need to know the best variety of annatto that will produce an adequate level of the bixin content, and develop a feasible techniques for harvesting, drying, packaging and storage of the seeds. If annatto seeds produced by our farmers can meet the quality requirements for local and export market, this may lead to increase agricultural production of the said commodity thereby gives an added income for the farmers.

At present, the export market has an annual demand of about 100-300 tons of annatto seeds. The Philippines may well be a potential supplier of seeds if the annatto farmers can be organized to provide consistent supply.

Future world - wide demand of annatto seeds and extracts is estimated to reach between 11,300 tons and 12,900 tons of seeds equivalent by 1995 and can absorb an additional 390 to 620 tons of seeds annually.

The overall action therefore, shows the future prospect of annatto to be encouraging especially in view of the increased utilization by the existing companies at need.

### **OBJECTIVES:**

1. To establish the index of maturity of the three (3) varieties of annatto
2. To establish drying parameters for maximum retention of bixin
3. To determine the packaging requirements of the dried seeds
4. To evaluate the storage stability of the seeds of annatto
5. To determine other qualities of the dried annatto seeds

### **REVIEW OF LITERATURE**

*Bixa orellana L.* also known as annatto, is named after Francisco de Orellana, the Conquistador who first explored the Amazon River in 1541. Its occurrence and cultivation were reviewed by Ingram and Francis (1969).

The annatto shrubs are abundantly grown in most tropical countries such as Brazil, Peru, Mexico, Ecuador, Dominican Republic, Jamaica, India, Sri Lanka.

Kenya and to a lesser extent, the Philippines, Turkey and Angola. The seeds of these shrubs are generally obtained from the third year after plantation, for a period of 10 to 12 years. The number of years vary from region to region depending on the variety of the plant, soil and climate. The average yield of annatto is between 300 kg and 600 kg/ha.

Annatto are usually traded as seeds. The highest quality seeds are exported from Jamaica and Caribbean Islands, with the bixin content of as high as 3%, compared with the 2% average for Central and South American suppliers. Seeds from the West Indies produce more red and less yellow pigments. Indian seeds however, contain a greater proportion of yellow than red pigments. The difference in quality and in the costs of production undoubtedly influence price levels. The most expensive annatto is coming from Jamaica and Peru and the cheapest from India.

Commercial annatto colors are also known as Orlean or Rocon. The FAO/WHO (1976) have defined specifications for the methods of production of these colors. Annatto extracts in oil, as solution or suspension, is prepared by extracting the outer coating of the seeds with the food grade vegetable oil. Solvent extraction is also employed. Solvents such as acetone, dichloromethane, ethanol, hexane, methanol, propanol and trichloroethylene can be used to extract the pigments. Aqueous annatto extracts are obtained by heating the pericarp, or the solvent extract thereof, with a solution of sodium or potassium hydroxide at 70°C or lower followed by filtration.

The major carotenoid of the pericarp extract *cis*-bixin, the mono-methyl ester of the dicarboxylic acid *cis*-norbixin (McKeown, 1961), is orange in color and insoluble in vegetable oil. It is readily converted on heating into a more stable *trans* isomer. *Trans* bixin is red in color and much more soluble in vegetable oil. Both *cis* and *trans* bixin, or stronger heating undergo a complex series of isomerization to produce principally a yellow pigment,  $C_{17}H_{20}O_4 \cdot CH_3CH_2CH_2OOC \cdot COOH$

Alkali treatment of *cis* and *trans*bixin produces the water soluble sodium or potassium salts of *cis* and *trans* norbixin.

Bixin and its isomers have maxima at about 500 nm and 470 nm in chloroform, whereas the yellow degradation pigment show maxima at about 404 nm and 428 nm and do not absorb at 500 nm (McKeown and Mark, 1962).

Van Esch *et al.* (1959) had an extensive metabolic studies on the major carotenoids of annatto. The annatto extracts used in the studies were manufactured in accordance with the FAO/WHO specifications without the use of solvents. The metabolic studies included work on acute metabolism in rat and man. This research study was conducted at the Unilever Research Laboratory at Colworth House, Bedford. This toxicological work was considered by the FAO/WHO (1970 a,b) resulting in an expanded monograph (FAO/WHO, 1976). The temporary acceptable daily intake (ADI) of 1.25 mg/kg body weight for annatto extracts has been given (FAO/WHO, 1970 a,b).

In rat, the oil soluble bixin and water soluble annatto pigments were de-

tected in the blood within a few hours after administering a single large oral dose. The level of pigments in the blood diminished during the following 24 hours, which suggest that the body is able to metabolize these pigments.

In man, annatto pigments were also detected in the blood after a single oral dose was taken, but within a few hours the blood level has fallen to zero. Only a trace of these pigments was found in the faeces. Therefore, both in rat and in man, annatto pigments are absorbed from the intestine into the blood and removal from the blood stream is quite rapid. It is apparent from these studies showing similar absorption from the gut and clearance from the blood of rat and man, that the rat is an appropriate test animal and that the results obtained by van Esch and others are relevant to the safety of annatto pigments in foods.

The general pattern of usage of annatto-based colors world-wide is estimated as follows: cheese making 50%; fish processing and maggot feed manufacture 20%; confectionery 10%; and other uses, including dairy products other than cheese and snacks, 20%.

## MATERIALS AND METHODS

Three (3) varieties of annatto were utilized in the implementation of the first objective of the study while in the succeeding objectives four (4) varieties were involved. The annatto varieties are the Indonesian White Flower (WF), Indonesian Pink Flower (PF), the Native (N), and the Yellow (Y) variety (Annex 1. 2, 3 & 4). The Yellow variety was not included in the establishment of the index of maturity because seeds of this var. were only available in Davao and in this regard, maturity cannot be monitored. The experimental field used in maturity evaluation was located at the National Crop Research and Development Center in Los Banos, Laguna. Seeds of each variety were harvested at different maturity period. The maturity days were monitored from the first day of the induction of flowers of annatto. At 45 days of maturity, the harvesting of the fruits started and every week thereafter up to the 60th day of maturity. Field observations were conducted as to the visual characteristics of annatto.

### Extraction of the Carotenoid Pigment

Bixin was extracted from annatto seeds by covering 1 gram of the seeds with 50 ml ethyl alcohol and stirred with the use of the magnetic stirrer. The mixture was then filtered and the extraction was repeated on the insoluble materials with the second 50 ml ethyl alcohol. Five extractions were done to remove all the coloring matter. The combined extracts were evaporated on a rotary evaporator at 40°C to about 50 ml. The dark red pigment was filtered off and dried in a vacuum oven.

The extracted pigment was purified by dissolving in ethyl alcohol, filtered and evaporated the solution and dried in a vacuum oven.

Following the same procedure, chloroform was also used as the extracting solvent.

### Characterization of the Purified Pigment

Since there was no available standard for bixin, the purified pigment was characterized and the results obtained were compared on data obtained from the literature available. This pigment was subjected to Ultraviolet Visible Spectrophotometer to determine the wavelength at which the maximum absorption occur. Further confirmation of the characteristics was done using Hitachi 270-30 Infrared Spectrophotometer to know the functional groups present in the pigment. Solubility tests were also conducted using different solvents such as petroleum ether, 0.1 N sodium hydroxide, vegetable oil, concentrated sulfuric acid and water.

### Spectral Assay of the Pigment

Spectrophotometric assay of the extracted pigment was done by dissolving 0.5 mg of the sample in chloroform, mixed and diluted to the mark in a 100 ml volumetric flask with chloroform. An aliquot of a 1 ml was taken and diluted to 100 ml. The absorbance was measured at 470 nm using LKB Biochrom UV-VIS Spectrophotometer. Using the value obtained, the total carotenoid expressed as bixin was calculated in terms of gram per 100 gram with the following formula:

$$\frac{A}{2826} \times \frac{100,000}{\text{wt.spl (mg)}} \times 100$$

### Physical, Chemical and Microbiological Analysis of Annatto

The Physical characteristics of the seeds were noted as the color of the pods changed during maturation. The sizes of the pods were measured and the average number of the seeds were recorded. The moisture content was determined oven drying. The water activity ( $A_w$ ) was also measured.

Crude fat and starch were analyzed using the American Association of Official Chemists (AOAC) methods. Bixin was evaluated by the spectrophotometric method. The microbial load of the seed samples were determined by taking the standard plate count and the yeast and mold counts. The standard plate on trypton glucose yeast agar was measured by preparing decimal dilutions of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  of the sample.

Potato dextrose agar acidified with 10% tartaric acid solution was the media used for the yeast and mold count.

### **Analysis of Commercial Annatto**

Six (6) commercial annatto seeds were examined for their physical, chemical and microbiological properties using the methods described earlier.

### **Harvesting of Annatto**

The capsule pods of annatto are bound in clusters. However, the pods in one branch do not mature at the same time, so harvesting of the pods were done by bunch. The matured bunch of pods were carefully cut off using a sharp clipper. The immature bunches left were harvested later when indications of maturity were evident.

The harvested pods were dehusked manually to separate the seeds from the hulls. The seeds were then transferred to a vibrating screen to remove the undesirable objects such as twigs, soil and other physical impurities.

### **Drying of Annatto Seeds**

Four (4) varieties of annatto seeds were used in the drying process. The seeds of the fourth variety, the Yellow (Y) var. were harvested in Davao National Crop Research and Development Center at Bago Oshiro, Davao City.

Seed samples from the four (4) varieties were simultaneously loaded in the fabricated solar dryer and in the cabinet type dehydrator. The solar dryer (Annex 5) with a capacity of 100-130 kg annatto seeds was fabricated in the laboratory. It is a rectangular box made of wooden frames with galvanized iron sheets. The top and the sides of the dryer are covered with transparent plastic which allows the radiation of the sun to penetrate inside. It has an upper and lower ventilation which allow dry cold air to enter and carry away moisture from annatto seeds. The removable drying trays are made of galvanized iron mesh wire that are laid over the wooden frames. The door on one side of the dryer is an easy access for the loading and unloading of the annatto samples. The base of the dryer is a black galvanized iron sheet which held a bed of rocks that keeps the heat inside the dryer. The temperature inside the dryer was measured using a glass thermometer. This was placed in the shaded area of the dryer and the temperature readings were taken without removing the thermometer. The mobile dryer was positioned facing East in the morning to its highest point in the midday. It was reversed in the afternoon to maximize the use of solar energy.

The other dryer used was a laboratory dehydrator which is provided with a fan. It has a capacity of 30-40 kg. During the drying process, samples from each variety were taken from different locations within the two dryers for analysis of moisture content, bixin, crude fat and starch after 8, 12, 16, and 24 hours of drying.

### **Packaging and Storage of the Dried Annatto Seeds**

After establishing the maximum bixin content of the annatto seeds dried in both dryers, the samples were packed in the 6"x 8" polyethylene bags. Each bag had a capacity of 500-gm seeds. These were stored at low temperature, room or ambient (28-32°C) and elevated (37°C) temperatures for six months. Analysis for moisture, water activity, bixin, crude fat, starch content and the microbial load of each variety were taken after storage.

### **RESULTS AND DISCUSSION**

The index of maturity of annatto was established by characterizing and evaluating the bixin content of the annatto seeds at different stages of maturity. The carotenoid bixin, the pigment obtained by ethyl alcohol extraction, is the major constituent of annatto and serves as an indicator of its quality. Comparison of the three (3) varieties of annatto showed differences in the physical characteristics. The harvested fruits of PF variety are of varied sizes. Three sizes were taken and characterized. Results indicate that there was no relationship between the variability of size and the composition of the seeds. The mean chemical contents of the three (3) varieties of annatto were not significantly different from each other. However, full development of the bixin content of the PF var. was found on the 50th day of maturity (Table 1), while the WF and N varieties showed the highest bixin content on the 55th day of harvest (Tables 2 & 3). Among the varieties, the WF showed the highest bixin content of 2.59%. The pigment obtained from all the varieties was the reddish orange color.

The WF variety had bigger size of seeds but the pods were smaller than the two varieties.

The seeds of the PF var. was found to have higher microbial load compared to the two varieties. Table 4 shows the data on the yield of the three varieties of annatto. These were based on the 4 x 4 m distancing of the tree per hectare. The WF var. had the highest seed recovery despite the smaller sizes of the trees.

### **Extraction and Evaluation of the Annatto Pigment**

The annatto pigment extracted by ethyl alcohol is a *cis*- and *trans*- bixin. It is a monomethyl ester of dicarboxylic acid. This is soluble in vegetable oil, dilute sodium hydroxide, petroleum ether and concentrated sulfuric acid but insoluble in water.

The color intensity of the purified bixin was measured spectrophotometrically. It gave a maximum absorption at 470 nm in chloroform (Figure 1). This method was used to evaluate the efficiency of the extraction method. The color of the annatto extracts in all varieties appeared to be the same.

The spectra of the purified bixin obtained from Infrared Spectrophotometer matched the spectra of the functional groups identified in the chemical for-

mula of bixin (Figure 11).

### **Analysis of Commercial Annatto**

A survey of annatto seeds available in different markets in Metro Manila and nearby provinces was done to evaluate their qualities. Different colors of the seeds were observed. The results of the quality assessment are shown in Table 5. The annatto seeds purchased in Divisoria market showed the highest bixin content of the survey samples but still below the standard requirement. The high moisture content and high water activity of the seeds from Balintawak market explained the presence of viable microbial cells detected from the samples.

### **Harvesting and Drying of Annatto Seeds**

The annatto seeds were dehusked manually to separate the seeds from the hulls. This method is used for small scale commercial operations. This was done carefully to prevent the pigment from sticking to the hands. All visible filth were removed.

Comparison of the solar dryer and the dehydrator showed that after 12 hours drying in the dehydrator, the standard bixin content was attained (Figure 3). The moisture content ranged from 8-9%. The low air flow with a moderate air temperature provided rapid drying. In solar dryer however, it took 16 hours to reach the maximum bixin content with the moisture content ranging from 7-9% (Table 6). Statistical analysis using t-test showed that the mean chemical contents of the four varieties of annatto are not significantly different.

### **Packaging and Storage of the Dried Annatto**

The shelf life of the dried annatto seeds which were held simultaneously at ambient (28-32°C) and elevated (37°C) temperature was evaluated for six (6) months. There was a slight fluctuation on the moisture content of samples held at ambient temperature. This may be due to the unstable relative humidity of the storage room. The bixin content remained stable up to the 4th month of storage (Figure 4). No marked differences were observed on the other chemical properties (Table 7). The minimal microbial load detected at ambient temperature is attributed to the alkaline property the carotenoid bixin (Table 9).

There was a gradual decrease on the moisture content from 9-6% of the samples stored at an elevated temperature. Bixin content also decreased (Figure 5). However, a slight increase on the crude fat content was observed in the Yellow var. (Table 8).

The polyethylene bags used in the packaging of the annatto was found to be appropriate. The polyethylene material of the bags add substantial protection

from the uptake of the water vapor of the annatto seeds. It protects the seeds from spillage and suited for transporting annatto in bulk because of its adequate strength. Aside from that, these packages are cheap and can be used several times.

### SUMMARY/CONCLUSION

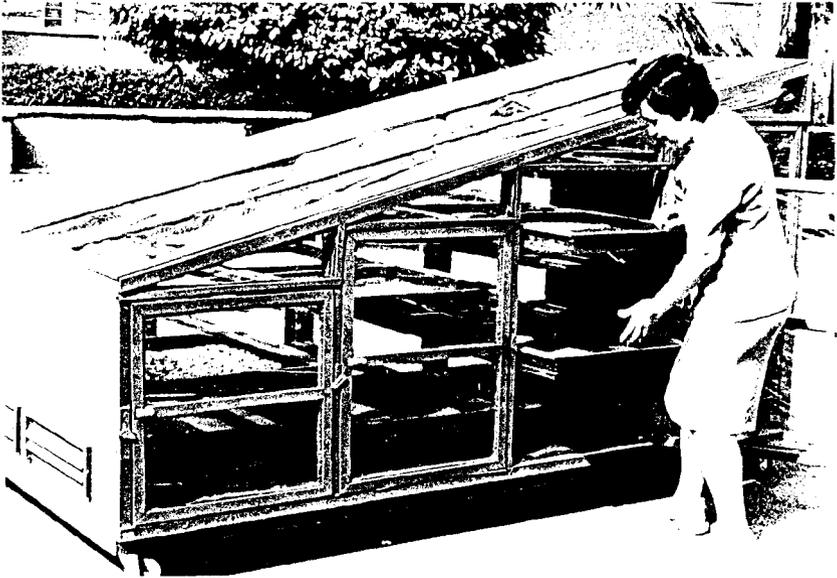
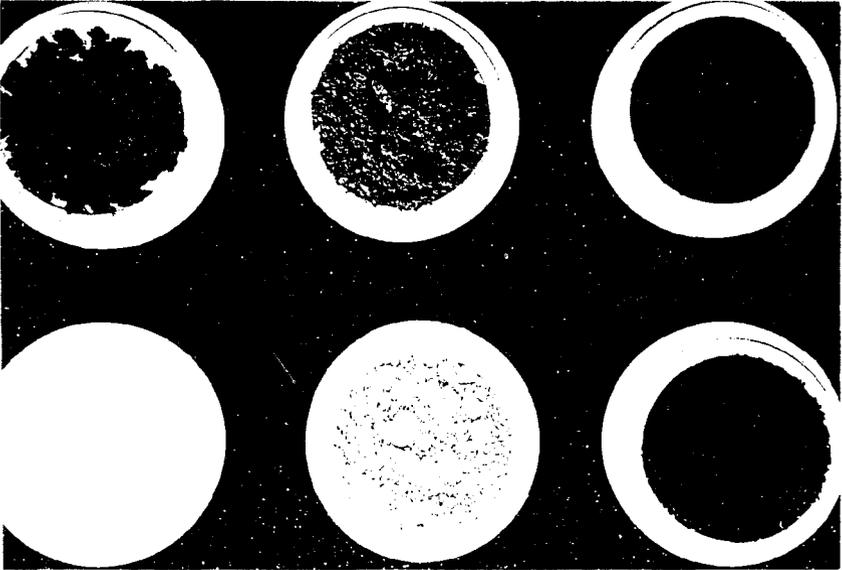
Carotenoid bixin is the major pigment of annatto. This is used as an indication of the quality of the annatto seeds. It is best extracted from the pericarp of the seeds by the use of ethyl alcohol or chloroform as solvents. Characterization of bixin gave a maximum absorption at 470 nm and the functional groups identified conformed with the functional groups present in the chemical formula of bixin.

The quality changes that took place during the growth of the annatto, the drying and storage give a valuable guide for proper handling and storage.

Drying of annatto by the use of solar dryer creates a physical environment which maintain the seed quality wherein the bixin content was retained at an acceptable level. Moreover, this method enhanced protection of the seed samples through dust and insect that constitute also to an improved and more consistent quality of the annatto seeds.

The solar dryer was economical compared to the dehydrator where the source of motive power is required. There is a considerable savings in energy costs. It can be constructed very easily from the materials that are available even in the rural areas.

Storing dried seeds using polyethylene bags as a packaging material resulted to the retention of bixin up to four (4) months storage at ambient temperature. Degradation of bixin was observed when the seed samples were held at an elevated temperature. With these results obtained, it is best to note that farm storage of the seeds can also be done to the extent that the air flow should be high with a low relative humidity of the storage atmosphere. The effect of the relative humidity is very important in the storage of the seeds.

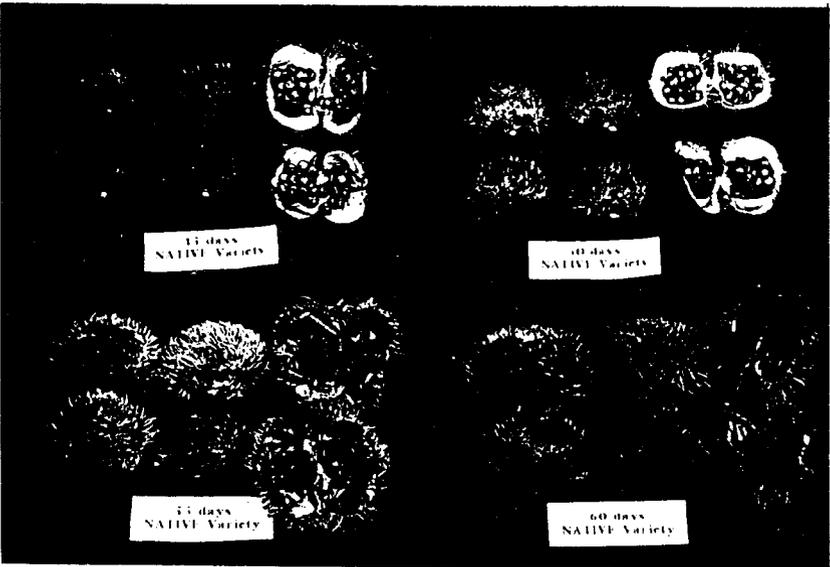
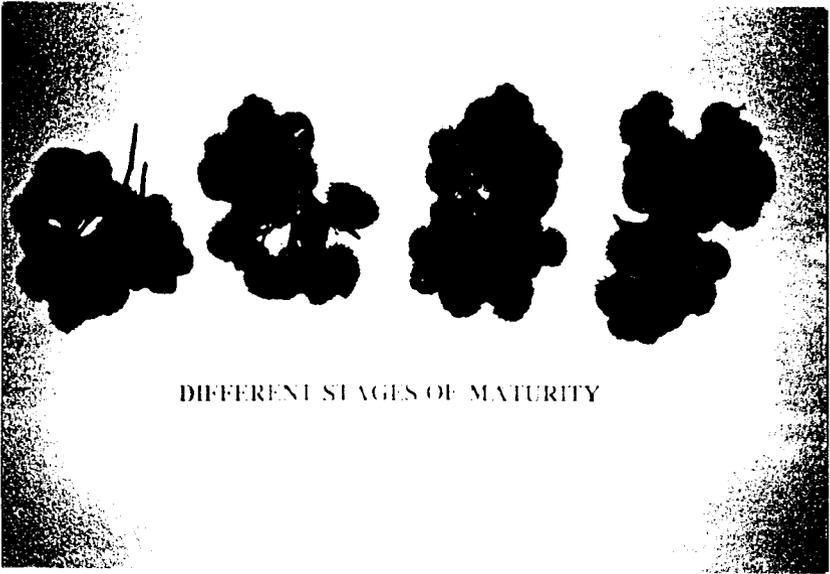


Solar Dryer

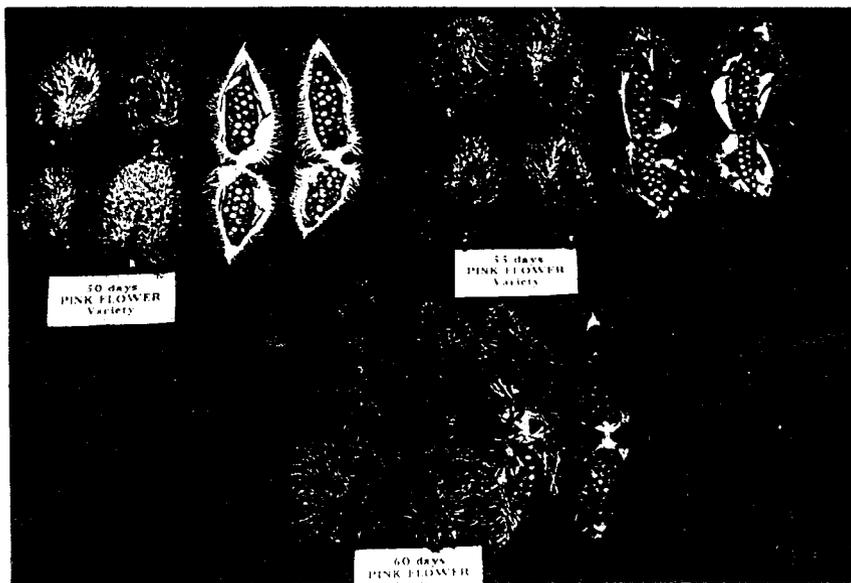


**Yellow Variety**

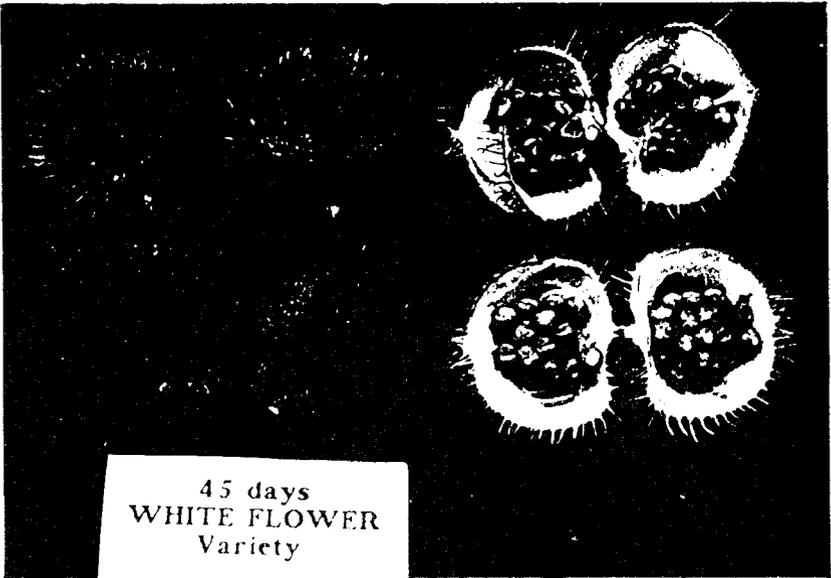
### Native Variety



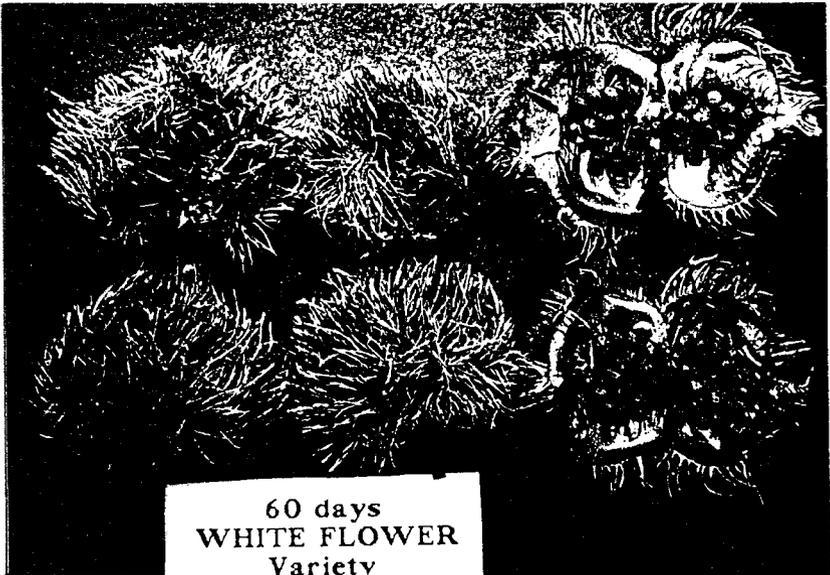
### Indonesian Pink Flower Variety



**Indonesian White Flower Variety**

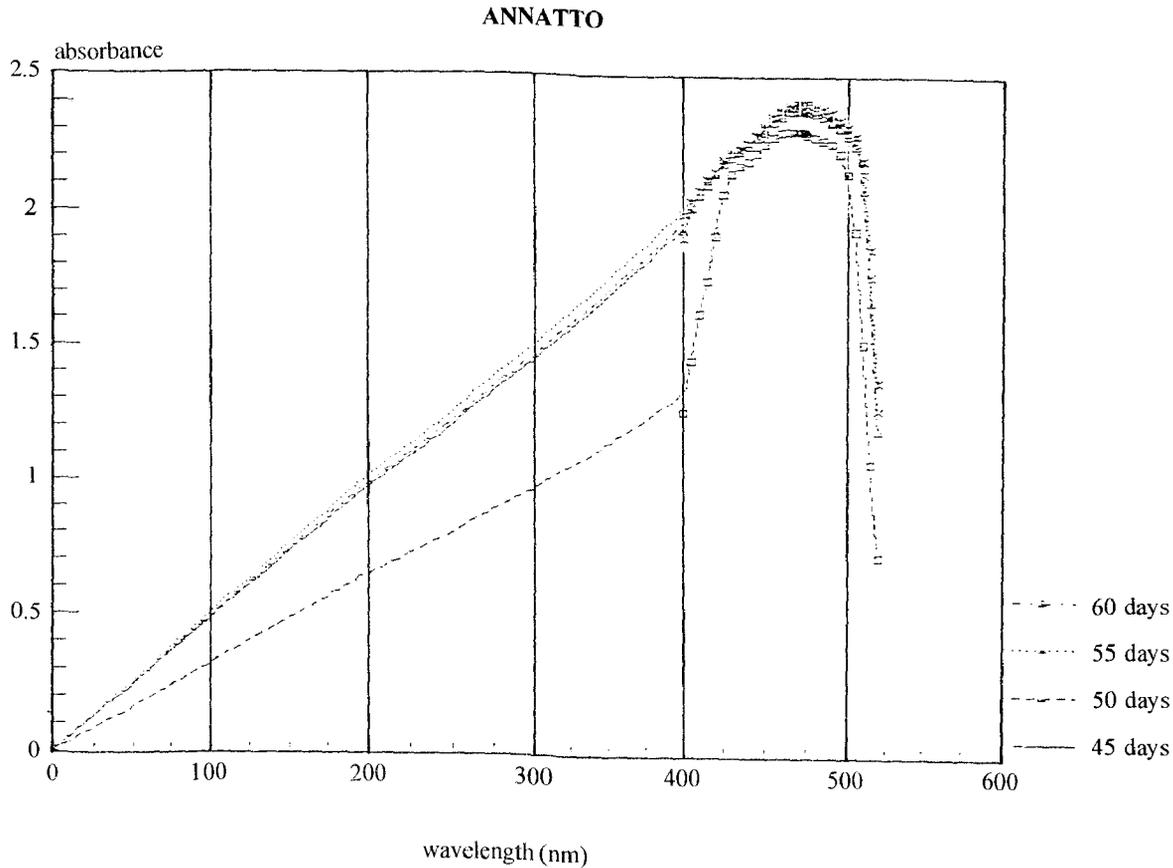


45 days  
WHITE FLOWER  
Variety



60 days  
WHITE FLOWER  
Variety

Figure 1.



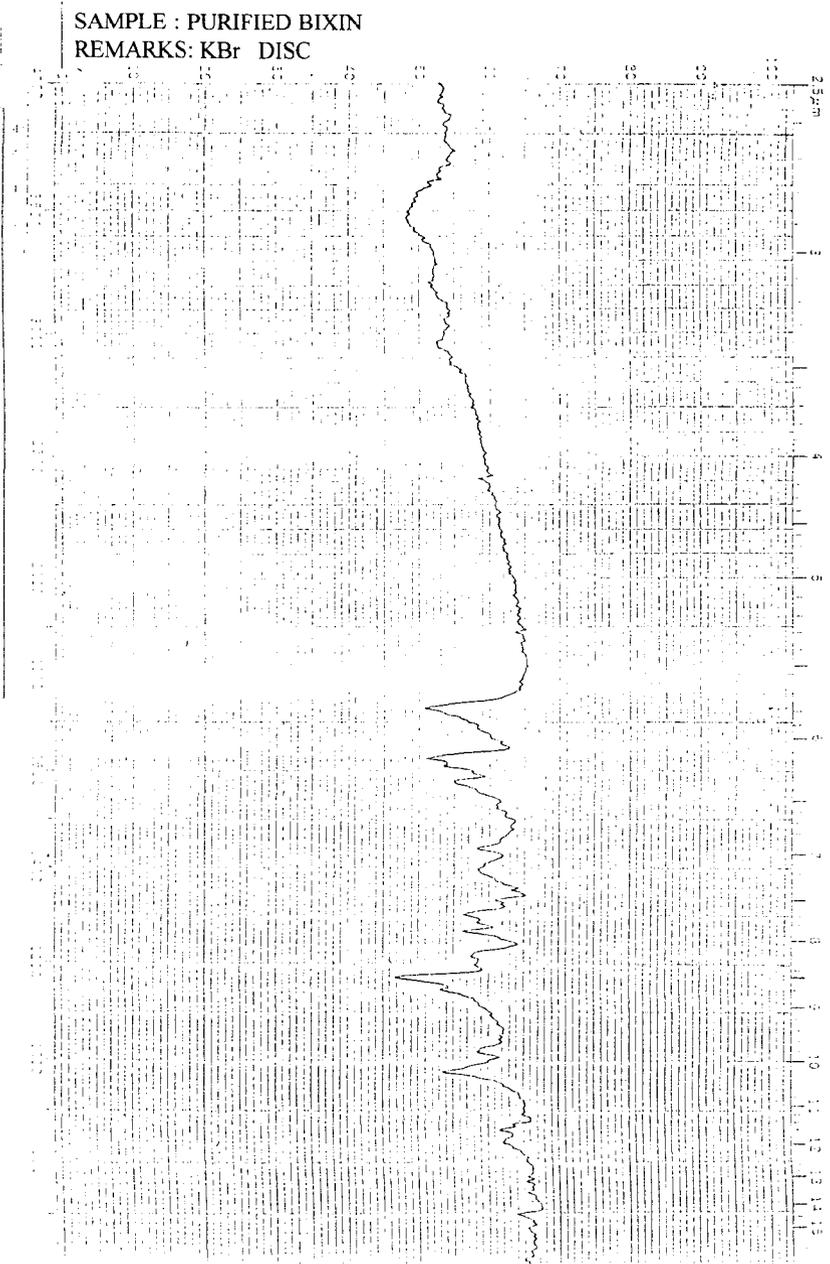


Figure 2.



Figure 3. Bixin content of the four(4) varieties of Annatto at different time of drying in solar dryer(a) and dehydrator(b).

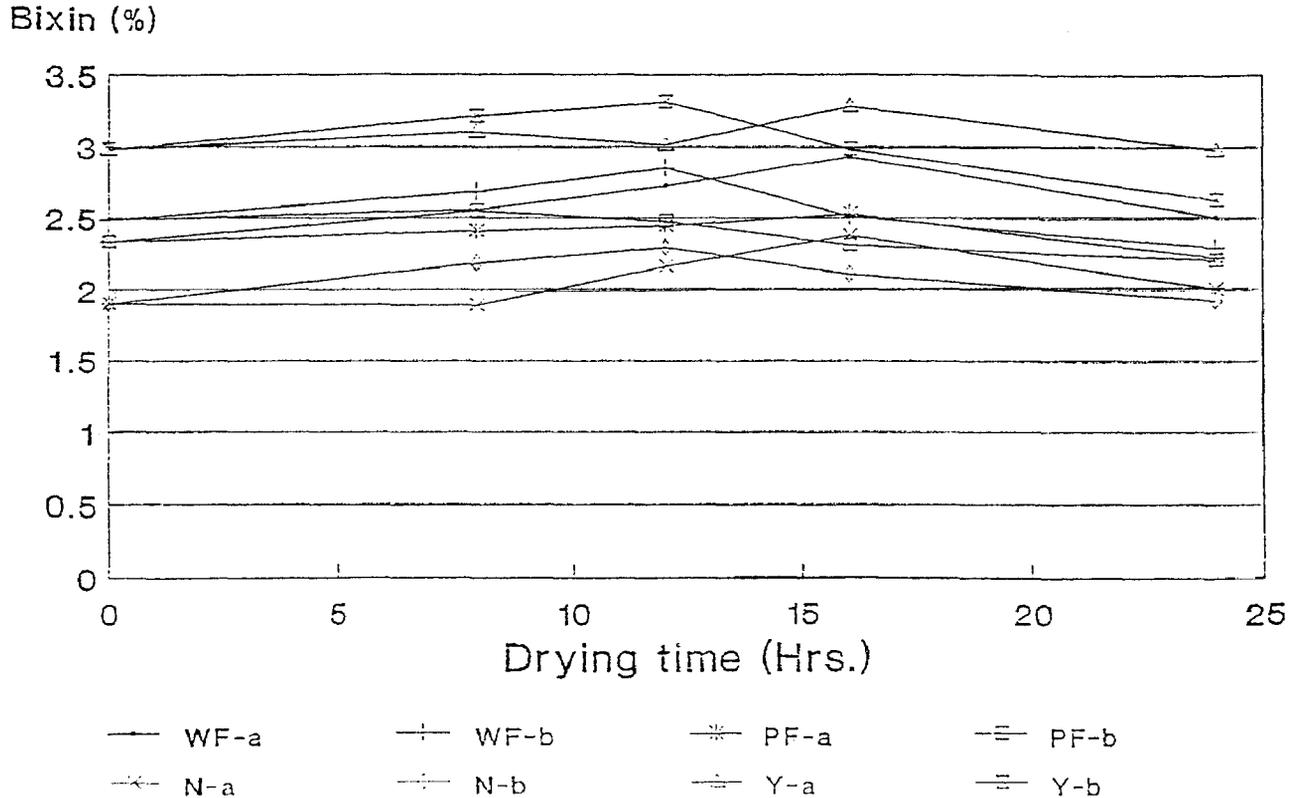


Figure 4. Bixin content of the four(4) varieties of Annatto stored in Ambient (28-32 Deg. C) temperature.

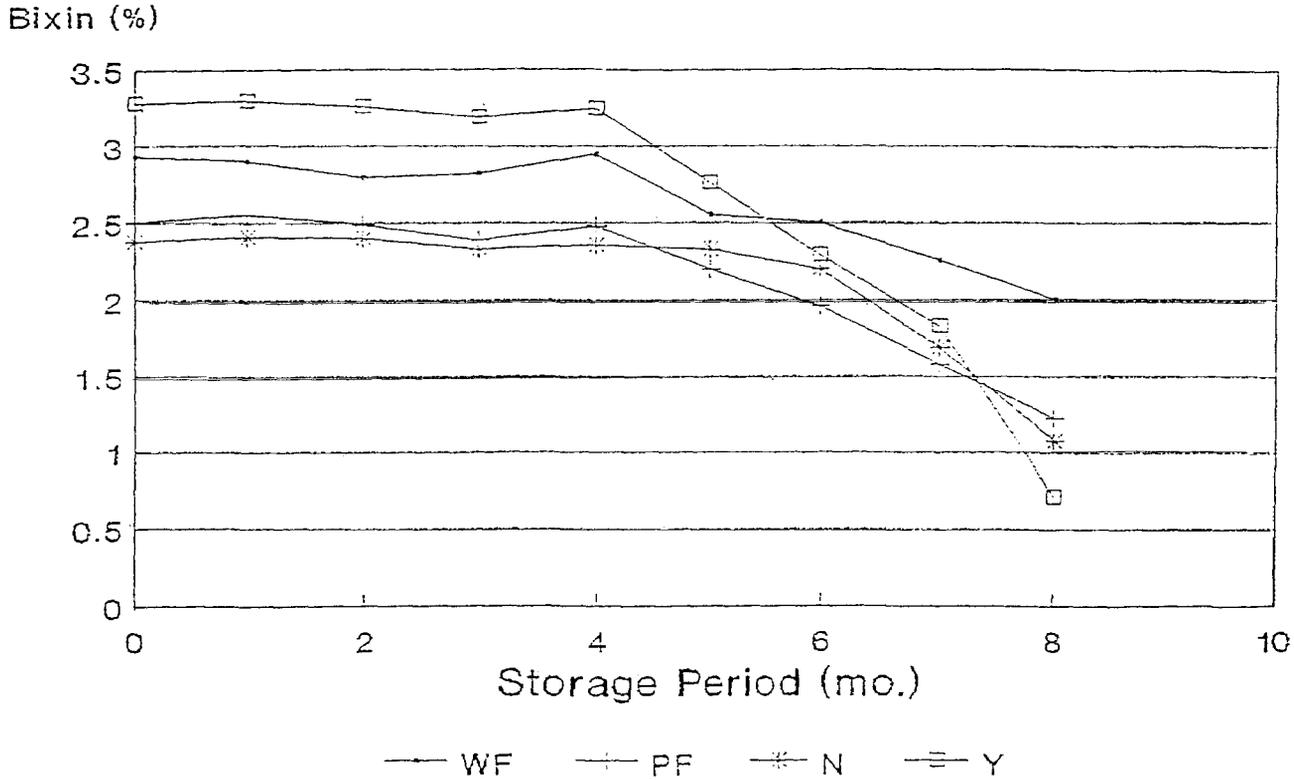
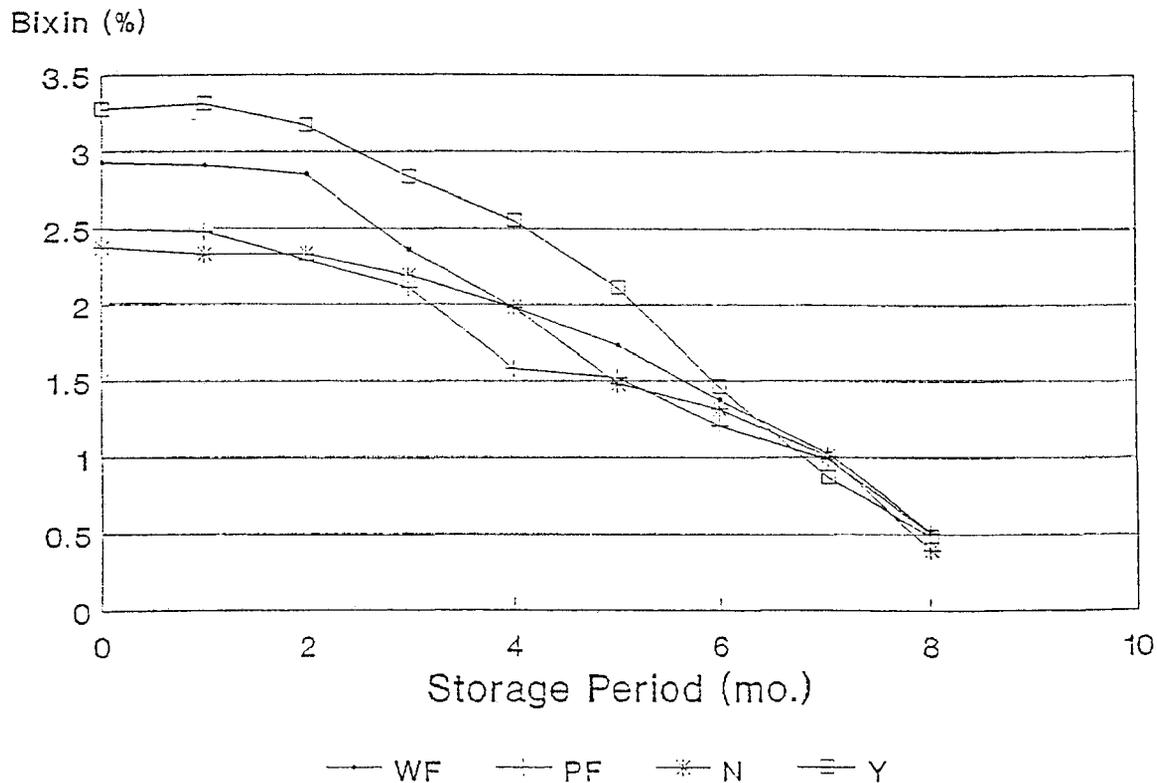


Figure 5. Bixin content of the four(4) varieties of Annatto stored at elevated (37 Deg. C) temperature.



**Table 1. Physico-chemical and microbiological analysis of the Indonesian Pink Flower Variety of Annatto at different stages of maturity.**

Maturity Period (Days)	Average Number of Seeds	Size of the Pods (mm)		Color of the Pods	Moisture (%)	Crude Fat (%)	Bixin (%)	Microbial Load	
		d*	L					Standard Plate Count (cells/ml)	Yeast & Mold Count (cells/ml)
45	50	30	45	red with red spikes	69.38	0.54	1.29	$3.2 \times 10^2$	$2.1 \times 10^2$
	50	30	50		72.63	0.63	1.03	$1.5 \times 10^3$	$2.5 \times 10^3$
	49	35	55		70.92	0.55	1.15	$1.9 \times 10^2$	$1.9 \times 10^2$
50	50	35	50	pale yellow with red spikes	69.22	0.48	2.54	$1.8 \times 10^3$	$1.2 \times 10^3$
	49	35	55		65.42	0.58	2.48	$2.1 \times 10^4$	$2.2 \times 10^3$
	50	40	60		70.71	0.47	2.16	$2.2 \times 10^3$	$3.1 \times 10^2$
55	50	35	50	light brown (non-splitted)	69.31	0.56	1.44	$2.0 \times 10^6$	$7.1 \times 10^4$
	50	40	55		60.88	0.48	1.68	$3.2 \times 10^7$	$6.5 \times 10^3$
	52	45	60		63.25	0.44	1.51	$5.3 \times 10^6$	$4.9 \times 10^4$
60	48	35	50	brown (splitted)	34.81	0.43	1.86	$2.0 \times 10^6$	$7.1 \times 10^4$
	53	40	55		42.56	0.32	1.53	$3.2 \times 10^7$	$6.5 \times 10^3$
	51	45	60		50.12	0.30	1.58	$5.3 \times 10^6$	$4.9 \times 10^4$

d\* taken on the biggest part of the pod

**Table 2. Physico-Chemical and microbiological analysis of the Indonesian White Flower Variety of Annatto at different stages of maturity.**

Maturity Period (Days)	Average Number of Seeds	Size of the Pods (mm)		Color of the Pods	Moisture (%)	Crude Fat (%)	Bixin (%)	Microbial Load	
		d*	L					Standard Plate Count (cells/ml)	Yeast & Mold Count (cells/ml)
45	29	30	35	green	75.36	0.39	1.05	$1.2 \times 10^2$	$1.9 \times 10^2$
50	35	35	40	greenish with red-dish spike	71.61	0.35	1.33	$2.1 \times 10^2$	$1.2 \times 10^2$
55	35	35	40	light green with pale red spike	65.31	0.42	2.59	$3.6 \times 10^4$	$1.3 \times 10^3$
60	38	35	40	brown (non-splitted)	54.53	0.48	2.16	$4.1 \times 10^4$	$2.1 \times 10^3$

**Table 3. Average results of the physico-chemical and microbiological analysis of the native variety of Annatto.**

Maturity Period (Days)	Average Number of Seeds	Size of the Pods (mm)		Color of the Pods	Moisture (%)	Crude Fat (%)	Bixin (%)	Microbial Load	
		d*	L					Standard Plate Count (cells/ml)	Yeast & Mold Count (cells/ml)
45	26	20	30	greenish with red-dish spikes	68.10	0.34	1.30	$2.0 \times 10^2$	$1.1 \times 10^2$
50	31	25	35	brown with greenish shade	50.18	0.40	1.84	$1.1 \times 10^3$	$2.2 \times 10^2$
55	34	25	35	brown (non-splitted)	23.24	0.88	1.94	$2.3 \times 10^4$	$3.1 \times 10^2$
60	34	25	35	brown (splitted)	19.10	0.37	1.69	$2.6 \times 10^4$	$4.5 \times 10^2$

**Table 4. Data on the yield of the three varieties of Annatto.**

	White Flower	Pink Flower	Native
Height (m)	2.00	2.50	5.00
Ave. Yield/tree (kg)	2.60	2.80	4.50
Seed Recovery/tree (%)	53.09	39.64	42.30
Yield/ha (kg)	1,625.00	1,750.00	2,812.00

**Table 5. Physico-chemical and microbial analysis of the commercial Annatto.**

Source of Sample	Color of The Seeds	Moisture (%)	Aw	Bixin (%)	Crude Fat (%)	Starch (%)	Microbial Load	
							Standard Plate Count (cells/ml)	Yeast & Mold Count (cells/ml)
Quiapo	red	14.78	0.68	1.16	0.22	34.75	7.1x10 <sup>5</sup>	8.3x10 <sup>4</sup>
Divisoria	brick red	15.32	0.74	2.13	0.25	35.37	1.6x10 <sup>6</sup>	6.4x10 <sup>4</sup>
Balintawak	orange	16.32	0.77	1.35	0.23	35.51	4.4x10 <sup>6</sup>	8.4x10 <sup>5</sup>
Dasmariñas	red	10.94	0.676	1.63	0.42	31.23	6.3x10 <sup>3</sup>	45(ESPC)
Binakayan	red	11.02	0.343	1.11	0.35	33.35	1.5x10 <sup>7</sup>	120(ESPC)
Tanauan	red	11.98	0.656	1.25	0.51	31.76	5.4x10 <sup>7</sup>	4.8x10 <sup>3</sup>
Lipa	brick red	10.70	0.316	1.81	0.53	33.04	8.6x10 <sup>6</sup>	6.6x10 <sup>2</sup>

**Table 6. Physico-Chemical Analysis of the four (4) varieties of Annatto at different time of drying in solar dryer<sup>a</sup> and dehydrator<sup>b</sup>.**

Drying period (Hrs.)	Variety	Moisture (%)		Aw		Bixin (%)		Crude Fat (%)	
		a	b	a	b	a	b	a	b
		0	WF	33.11	33.11	0.861	0.861	2.49	2.49
	PF	23.65	23.65	0.838	0.838	2.33	2.33	0.55	0.55
	N	34.56	34.56	0.807	0.807	1.90	1.90	0.40	0.40
	Y	14.71	14.71	0.566	0.566	2.98	2.98	0.41	0.41
8	WF	13.21	11.40	0.536	0.511	2.56	2.68	0.21	0.56
	PF	12.32	12.07	0.522	0.543	2.41	2.50	0.19	0.67
	N	13.82	12.11	0.562	0.554	1.89	2.18	0.17	0.58
	Y	11.21	10.83	0.522	0.479	3.10	3.21	0.36	0.53
12	WF	10.89	9.29	0.502	0.463	2.72	2.85	0.33	0.59
	PF	9.73	9.92	0.457	0.518	2.45	2.48	0.20	0.38
	N	10.31	9.18	0.512	0.451	2.16	2.29	0.42	0.44
	Y	9.23	8.11	0.469	0.409	3.01	3.31	0.38	0.48
16	WF	9.11	8.33	0.432	0.411	2.93	2.51	0.40	0.83
	PF	9.56	8.84	0.455	0.419	2.50	2.31	0.36	0.41
	N	8.69	8.64	0.412	0.410	2.38	2.10	0.41	0.46
	Y	7.98	7.63	0.398	0.403	3.28	2.98	0.48	0.53
24	WF	7.64	7.32	0.415	0.374	2.51	2.32	0.48	0.91
	PF	8.19	7.20	0.426	0.377	2.23	2.21	0.45	0.46
	N	7.83	7.51	0.422	0.389	2.01	1.93	0.44	0.51
	Y	7.50	7.03	0.408	0.351	2.98	2.63	0.66	0.44

**Table 7. Analysis of the four (4) varieties of Annatto stored at ambient temperature (28°C).**

Analysis	Variety	Storage Period (Months)									
		0	1	2	3	4	5	6	7	8	
Moisture (%)	WF	9.11	9.88	10.09	8.36	8.18	10.16	7.67			
	PF	9.56	10.60	11.40	10.04	11.78	11.02	10.76			
	N	8.69	10.29	10.28	9.59	11.40	10.06	7.81			
	Y	7.98	10.36	10.58	9.66	12.93	9.67	10.91			
Aw	WF	0.432	0.458	0.460	0.408	0.400	0.469	0.387			
	PF	0.455	0.489	0.492	0.439	0.518	0.501	0.479			
	N	0.412	0.471	0.471	0.453	0.508	0.446	0.392			
	Y	0.398	0.463	0.483	0.461	0.535	0.455	0.489			
Bixin (%)	WF	2.93	2.90	2.79	2.82	2.95	2.55	2.50	2.26	2.01	
	PF	2.50	2.55	2.49	2.39	2.48	2.20	1.96	1.58	1.23	
	N	2.38	2.41	2.40	2.33	2.36	2.33	2.20	1.69	1.08	
	Y	3.28	3.30	3.26	3.19	3.24	2.76	2.29	1.83	0.71	
Stryach (%)	WF	34.47	34.71	41.56	36.23	34.96	36.56	41.00			
	PF	32.09	31.42	42.33	41.95	36.11	33.89	33.55			
	N	37.87	35.58	40.86	41.83	37.71	38.24	38.69			
	Y	33.12	32.30	31.70	35.52	34.26	32.89	30.24			
Crude Fat (%)	WF	0.40	0.34	0.38	0.38	0.56	0.53	0.67			
	PF	0.36	0.41	0.35	0.81	0.52	0.62	0.68			
	N	0.41	0.28	0.21	0.60	0.27	0.33	0.48			
	Y	0.48	0.68	1.01	0.98	1.11	1.18	1.11			

**Table 8. Physico-chemical analysis of the four (4) varieties of Annatto stored at elevated temperature (37°C).**

Analysis	Variety	Storage Period (Months)								
		0	1	2	3	4	5	6	7	8
Moisture (%)	WF	9.11	9.51	9.58	9.22	8.86	7.88	6.61		
	PF	9.56	9.37	8.97	9.31	10.46	9.16	8.14		
	N	8.69	9.32	8.52	8.82	9.48	7.39	6.22		
	Y	7.98	8.10	8.11	7.69	7.55	7.42	6.99		
Aw	WF	0.432	0.451	0.456	0.441	0.439	0.401	0.328		
	PF	0.455	0.444	0.448	0.446	0.459	0.458	0.412		
	N	0.412	0.441	0.436	0.438	0.451	0.379	0.299		
	Y	0.398	0.401	0.411	0.386	0.399	0.383	0.341		
Bixin (%)	WF	2.93	2.91	2.85	2.36	1.98	1.73	1.36	1.03	0.52
	PF	2.50	2.48	2.29	2.11	1.58	1.52	1.21	0.99	0.51
	N	2.38	2.33	2.33	2.19	1.98	1.48	1.32	1.01	0.40
	Y	3.28	3.31	3.17	2.83	2.51	2.11	1.46	0.87	0.49
Strach (%)	WF	34.47	34.42	37.89	41.72	39.99	38.83	41.14		
	PF	32.09	33.89	40.85	40.15	39.17	39.64	35.98		
	N	37.87	31.08	33.42	33.21	36.58	35.57	37.20		
	Y	33.12	32.37	32.92	34.79	36.88	35.56	36.11		
Crude Fat (%)	WF	0.40	0.18	0.41	0.48	0.25	0.74	0.66		
	PF	0.36	0.41	0.39	1.06	1.08	1.10	0.98		
	N	0.41	0.30	0.09	0.49	0.21	0.33	0.56		
	Y	0.48	0.51	0.66	0.73	0.92	1.03	1.11		

**Table 9. Microbiological analysis of four (4) varieties of Annatto stored at ambient (28-32°C) and elevated (37°C) temperatures.**

Storage Period (mo.)	°C	M I C R O B I A L L O A D							
		Standard Plate Count (cells/ml)				Yeast and Mold Count (cells/ml)			
		Variety				Variety			
		WF	PF	N	Y	WF	PF	N	Y
0		$1.7 \times 10^6$	$2.1 \times 10^6$	$1.2 \times 10^4$	$2.8 \times 10^5$	55(ESPC)	$5.0 \times 10^3$	135(ESPC)	$1.9 \times 10^3$
1	c	$2.3 \times 10^5$	$1.3 \times 10^6$	$8.4 \times 10^5$	125(ESPC)	$7.5 \times 10^2$	$1.2 \times 10^3$	85(ESPC)	<10(ESPC)
	d	$2.4 \times 10^5$	$1.7 \times 10^6$	$1.4 \times 10^7$	30(ESPC)	<10(ESPC)	50(ESPC)	<10(ESPC)	<10(ESPC)
2	c	$3.6 \times 10^4$	$1.5 \times 10^7$	$7.6 \times 10^3$	<10(ESPC)	50(ESPC)	$4.8 \times 10^2$	30(ESPC)	65(ESPC)
	d	$4.6 \times 10^4$	$3.3 \times 10^5$	$4.8 \times 10^4$	<10(ESPC)	<10(ESPC)	60(ESPC)	<10(ESPC)	30(ESPC)
3	c	$4.8 \times 10^4$	$6.6 \times 10^4$	$1.9 \times 10^3$	$1.1 \times 10^3$	<10(ESPC)	170(ESPC)	<10(ESPC)	<10(ESPC)
	d	$1.6 \times 10^4$	$5.0 \times 10^4$	$2.0 \times 10^3$	$1.0 \times 10^2$	$4.3 \times 10^3$	<10(ESPC)	<10(ESPC)	<10(ESPC)
4	c	$1.7 \times 10^4$	$3.3 \times 10^2$	$1.8 \times 10^3$	235(ESPC)	<10(ESPC)	<10(ESPC)	<10(ESPC)	<10(ESPC)
	d	300(ESPC)	$2.8 \times 10^6$	$1.9 \times 10^3$	200(ESPC)	<10(ESPC)	<10(ESPC)	$3.1 \times 10^2$	<10(ESPC)
5	c	$1.3 \times 10^3$	$6.3 \times 10^2$	<10(ESPC)	<10(ESPC)	<10(ESPC)	<10(ESPC)	<10(ESPC)	<10(ESPC)
	d	$9.5 \times 10^3$	$7.3 \times 10^3$	$9.6 \times 10^3$	$4.6 \times 10^3$	<10(ESPC)	40(ESPC)	<10(ESPC)	<10(ESPC)
6	c	<10(ESPC)	$1.1 \times 10^3$	160(ESPC)	<10(ESPC)	30(ESPC)	$40.0 \times 10^4$	340(ESPC)	<10(ESPC)
	d	$8.4 \times 10^2$	$6.1 \times 10^3$	315(ESPC)	<10(ESPC)	380(ESPC)	$7.0 \times 10^3$	$5.78 \times 10^4$	$1.28 \times 10^2$

# SUGAR QUALITY SPECIFICATIONS, SUGAR HANDLING, STORAGE AND DISTRIBUTION

*Agnes G. Collado and Nora I. Chinjen  
Sugar Regulatory Administration, Department of  
Agriculture, Philippines*

## ABSTRACT

Cane sugar, in commercial application, generally refers to sucrose or saccharose. Its most important use is as a basic food. Sugar is one of the best and cheapest sources of energy in terms of calories per unit cost. However, sugar is usually combined with other food to increase its nutritional value, improve the taste and keeping qualities. The properties of sucrose in foods important to food technologists and the various types of sugar are described.

Manufacturers are concerned with product shelf life and acceptance, and consumers mostly with product quality. Sugar is produced in different grades for specific uses. It is manufactured under rigid and controlled conditions and also subjected to quality tests. Standards for raw and white sugars are presented and discussed.

Although sugar is not a perishable product, difficulties are encountered in the physical handling of the product. The methods of handling, sugar distribution and the requirements for proper storage are discussed.

The sugar of household and industry generally refers to sucrose and saccharose. In our country, it is produced from the sugarcane plant, a tropical grass belonging to the same tribe (*Andropogoneae*) as sorghum and corn (maize). The modern sugarcane plant is a complex hybrid of two or more of the five species of the genus *Saccharum*.

The two main constituents of the sugarcane plant are sucrose in the juice and cellulose in the fiber. The simple sugars glucose and fructose also occur unbound in sugarcane, usually in lesser amounts than sucrose. The production of sugar from cane juice is based on sucrose recovery or the ability of sucrose to crystallize from a thick syrup while glucose and fructose remain in solution.

## SUGAR MANUFACTURE

### Raw Sugar

The manufacture of sugar involves a number of operations which in themselves constitute two industries (milling and refining) 3. The raw sugar processing operation is divided into various stages, namely: preparation and milling of canes (extraction); clarification; evaporation; crystallization and centrifugation.

First, the cane is weighed and prepared for grinding by revolving knives and hammer mill shredders. Then, it is crushed by heavily grooved crusher rolls extracting a large part of the juice. To aid in the extraction, sprays of water or thin

juice are directed on the bagasse as it emerges from each mill. The by-product of the process, bagasse, often goes to the boilers as fuel.

The dark green juice from the mills is filtered to remove suspended solids and weighed. It is then clarified with lime and heated to remove the maximum amount of both soluble and insoluble impurities. The mud which separates from the clear juice by sedimentation is filtered off on rotary drum vacuum filters. It is discarded or goes to the field as fertilizer or soil ameliorant.

The clarified juice goes to the multiple effect evaporators where about 90% of the water is removed. The syrup then goes to single effect vacuum pans where it is further evaporated until saturation. At this point, seed grains are added to serve as nuclei for sugar crystallization. More syrup is added as the water evaporates. When the crystals and syrup from a dense mass known as massecuite, the contents of the pan is discharged into a crystallizer.

The massecuite from the crystallizer is drawn into a revolving machine called centrifugal. The basket of the centrifugal, suspended on a spindle, has perforated sides lined with wire cloth and perforated metal sheets. As the basket revolves, the molasses passes through the perforated lining while the sugar crystals are retained. The molasses which contains approximately 30% sucrose, 20 reducing sugars and ash, organic non-sugars and water is used as an ingredient for cattle feed, raw material in the manufacture of industrial alcohol, yeast and others.

### White Sugar

The refining process consists of the following stages: affination, defecation or clarification, decolorization, evaporation, centrifugation, and drying. Affination or washing is the first step in sugar refining. The process involves mingling with a warm barely undersaturated syrup to loosen the molasses film: purging of the mixture in the centrifugals and washing with hot or cold water or a high purity sweet water after separating the syrup.

The washed raw sugar is dissolved with water in a melter and clarified or defecated after pre-screening, to remove coarse materials, using lime and either phosphoric acid or carbon dioxide ( $\text{CO}_2$ ). Clarification removes insoluble materials (bagacillo, soil, suspended solids) and colloidal matter.

The principal control parameter in a refinery is color. The various types of colourants are phenolics, melanoidins, caramels and invert degradation products. Color is removed usually with carbonaceous adsorbents made from naturally occurring materials such as bone char, granular or powdered carbon and ion exchange resins.

The syrup which has been decolorized to an almost water white solution goes to multiple effect evaporators for preconcentration to about 76° Brix. The liquor from the evaporators goes to vacuum pans for further boiling. Seed grains are added to serve as nuclei for the sugar crystals. When the pan is full, the massecuite is dropped to crystallizers for curing (by cooling) and then to centrifugals for purging. The moist sugar discharged from the centrifugal machines

is conveyed to dryers for drying.

## PROPERTIES OF SUCROSE

The most important use of sugar is as a basic food. It is one of the best and cheapest source of energy in terms of calories per unit cost. Sugar is used-with other foods as a sweetener or as a means of improving and keeping qualities. A great part of the sugar produced is utilized in the food industries. The following are the physical and chemical properties of sugar (9) which makes it an essential ingredient in the manufacture of a wide variety of food products:

### 1. Sweetening and preservative agent

Sugar by virtue of its sweetness is highly accepted as an ingredient in food. It is also used as a preservative in various products notably, jams, jellies preserves and candied or glazed fruit.

### 2. Texture or body

Sugar provides body or texture in many food products which enhances their appeal.

### 3. Flavor blender, accentuator, modifier

When used in subthreshold level, sugar causes subtle flavor changes. It can also blend and accentuate flavor when added to certain food.

### 4. Dispersing agent

Sugar is widely used as a dispersing agent in food mixes because of its ability to break-up or separate the various ingredients resulting in more uniform mixtures.

### 5. Lubricant

Sugar, in both syrup and dry forms, improves blending during mixing and baking of batters and other mixtures.

### 6. Caramelization

When sugar is heated to about 175°C, it melts and the color changes from white to brown. The product formed is called caramel and this contributes to the

brown color of baked goods. Caramel is a major food colorant and flavoring agent.

## TYPES OF SUGAR

In many countries, several types of sugar are available to meet the particular requirements of various products. Some of these are:

1. **Large grain granulated sugars** - These are typically graded as standard, medium, medium fine and sanded and largely used by manufacturers of cordials and liquors.

2. **Regular granulated sugars** - These constitute a large part of refined sugar produced and are classified as extra fine, used in bakery products, beverages, dairy products; fruit granulated; used mainly in dry mixes and Baker's special.

3. **Confectioner's sugar** - contains cornstarch to prevent caking and designated at 6x, 10x, or 12x to indicate degrees of fineness.

4. **Brown (soft) sugar** - made by boiling fine crystals from purified and relatively low purity syrups. The crystals are covered with a layer of colored molasses flavored syrup giving it the unique flavor and color of brown sugars.

5. **Liquid sugars** - the two basic types are: (a) liquid sucrose-containing about 67% solids and is made from melted granulated sugar or from high grade refinery process liquors that have been treated with char or carbon without prior crystallization or purified by a mixed-bed ion exchange process to further reduce ash and color contents, and (b) liquid invert sugar containing 52-58% reducing sugars at 76° brix and is produced by acid inversion of melted granulated sugar and filtration with powdered carbon to improve color and clarity or from high grade char or carbon treated process liquors which were further processed with a two-bed (cation-anion) ion-exchange resins.

6. **Cubes and tablets** - which are pressed or molded sugars.

## SUGAR QUALITY SPECIFICATIONS

Consumers be they manufacturers or end users are all concerned about product quality.

Manufacturers are specially concerned with product shelf life and purity.

Sugar is manufactured under rigid and controlled conditions subject to quality tests. A quality specification or standard is established for a particular commodity to assure consumers of a sound, wholesome and unadulterated product. The standard specifications for sugars were established to serve as authoritative standards and guides for producers, traders/ distributors and end users. The specifications include descriptions, requirements for quality, purity, methods of analysis, packaging, etc. The specifications were based on the highest level of purity available at competitive prices, while the requirements relating to food contaminants, additives, and microflora were expressed in terms of maximum permissible limits. Methods of analysis were provided for uniform determination of conformity. However, needs and preferences of consumers change as they are exposed to improvements in product quality and technology. The present standards for raw and refined sugar were revised to reflect these preferences taking into account the technological developments during the last twenty six years.

### RAW SUGAR

The standard specification for Raw Sugar 11, designated Philippine Standard No. 061-01: 1976, was established in 1976 to assess and control the quality of Philippine raws. At that time, raw sugar was manufactured mainly for export. Reference to CODEX Alimentarius, International Commission for the Uniform Methods of Sugar Analysis (ICUMSA), Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC), Philippine Sugar Institute Rules and Regulations of the Sugar Laboratory, the Official Methods of Chemical Control (OMCC) and US Contract No. 10 (Bulk raw sugar contract of the American Sugar Company) have been made in order to develop specifications acceptable to both local and foreign markets. In 1980, the standard was revised (12) upon the request of the sugar millers to meet the changing demands of these markets. In this revised standard, raw sugar was categorized into two types, namely: centrifugal raw sugar and washed sugar. The maximum level for Safety Factor was also increased to 0.27.

The United States is the major importer of Philippine raw sugar. Its incentive scheme for raw sugar quality is based on standards set by American refiners and consumers or users. The Domino Sugar Company (formerly American Sugar Company), the biggest sugar refiner in the United States, established the criteria of quality for imported sugar in 1968. It has since been internationally adopted as U.S. Contract No. 10 (now the Domino Raw Sugar Contract) and was revised (7) in 1984. The specifications indicated in both contracts cover the most significant characteristics that affect the refining and keeping qualities of raw sugar. However, in the new contract, the following modifications were made:

- 1) factors for deriving maximum and minimum ash content were changed,
- 2) maximum and minimum levels for grain size were changed from 55 and 20 to 52 and 22, respectively,

- 3) color was specified not only for raw but also for refined sugars,
- 4) inclusion of dextran.

Over the past years, raw sugar exported to the United States were of variable quality. Penalties amounting to millions of dollars were imposed on color, grain size, ash and dextran. The specification standards for Philippine raw sugars had to be revised again in order that our export raw sugar will stand competition in the foreign markets. The Sub-Committee on Sugar under the Technical Committee on Agricultural and other Food Products of the Bureau of Product Standards was organized to undertake the revision of the raw as well as the refined sugar specifications. All sectors of the industry and consumers were represented in order to develop standards that are practical, realistic, impartial and acceptable to both the sugar industry and end users. Maximum levels for dextran and lead were included in the revised specifications for raw sugar. Dextran was included because of its harmful effects in sugar processing resulting in reduced overall recovery and poor sugar quality. Two maximum permissible levels for lead were also included. The level, 0.5 mg/kg is for raw sugar intended for direct consumption. In most countries, sugar is directly consumed as white sugar. However, in the Philippines, brown sugar is still preferred by a majority of consumers. If the sugar is intended for refining, the level 1.0 mg/kg is allowed.

## WHITE SUGAR

In 1976, the standard specification for refined sugar (13) designated Philippine Standard No. 061-02; 1976, was established to define the quality of a refined sugar that will conform with the requirements of foreign and domestic markets. The standard covered the most significant quality characteristics which could influence or affect its use and processability. Sugar was categorized into two grades, namely: premium and standard refined. Reference to CODEX Alimentarius, International Commission for Uniform Sugar Analysis (ICUMSA) and Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) were made.

The standard was revised (15) in 1992. The revision was made to meet current demands, preferences and requirements of consumers or users taking into account the prevailing sugar quality. Analysis of refined sugars I from refining years 1978 to 1993 show that the quality of Philippine standard refined sugar is consistently above the old specifications. In the revised standards, refined sugar was designated white sugar and requirements for refined as well as mill/factory white sugars were specified. White sugar was further classified according to their characteristics into the following grades: refined bottlers, refined premium, refined standard and mill/factory white. The sugar industry has traditionally relied heavily upon the production of raw sugar for export to the United States. However, with the drastic reduction of the U.S. quota, fluctuating world

market prices for raw sugar and the increase in demand for refined sugar, certain producers turned to the manufacture of direct consumption sugar or mill/factory white sugar. This sugar is produced by direct boiling of concentrated cane juice after treatment with sulfur dioxide, carbon dioxide and lime without first making a raw sugar to be remelted for refining. It is adequate for household and other industrial uses where the quality requirements do not justify the higher cost of refined sugar.

Sugar varies in quality and should be produced according to specific use. The domestic market for refined sugar has increased considerably due to increased direct consumption and growth in the food, beverage and pharmaceutical industries. Grading is vitally important in this market. The manufacture of these products require precise formulations. These products need specific sugars with exacting standard of grades without any variations in quality. For example, the beverage industry is one of the major user of sugar in the Philippines. It requires a bottler's standard grade sugar which is probably the highest grade that can be produced by any refinery. Any variations in sugar quality that affects beverage quality is of great concern not only to the bottlers but also to the sugar producers or refiners.

Maximum levels for  $\text{SO}_2$  content (10) were included for all grades of sugar. Mill/factory white sugar contains residual  $\text{SO}_2$  (as  $\text{CaSO}_3$ ) which maybe occluded within the sugar crystals or present in the syrup film covering the crystals. In many countries, the level of  $\text{SO}_2$  in food is regulated by food laws. Sugars containing 10 parts of  $\text{SO}_2$  per million is still suitable for table use but not for beverage, canning or other industrial use.

A maximum level of total thermophilic spores count for bottlers and premium grades was also included because while refined sugar had not been previously considered as a source of thermophilic flora, later investigations showed the presence in (or on) the sugar as adventitious contaminants. This is of great importance especially to the canning industry where the spores of thermophilic spoilage bacteria are so resistant that they may survive the most rigid processing temperatures, especially in non acid products.

Since the focus of the standards is also on consumers health protection, maximum permissible levels for the toxic heavy metals such as Arsenic, Lead and Copper were also included. The concentration of these metals in sugar or food is already limited by international standards given by the CODEX Alimentarius Commission.

## SUGAR HANDLING AND STORAGE

### Raw Sugar

Raw sugar is generally shipped and stored in bulk. Bulk handling enables savings on bags and transport, reduces handling cost and time at mills, ports and refineries. For bulk storage, calculations maybe based on a bulk density of 55 lb/

cu.ft. The angle of repose is about 40° which maybe increased to as high as 50° for moist sugar. The angle of slide is about 35° and the angle of draining is 55°. The Philippines has a complete system of bulk terminals and handling ports to handle the sugar production. The bulk terminals are located at Pulpandan, Negros Occidental and Guimaras Island, Iloilo while the loading ports are at Tolong and Amlan, Negros Oriental; San Carlos, Negros Occidental and Ormoc, Leyte.

Unless the raw sugar is immediately processed in an annexed refinery after production. It should first have the proper keeping qualities to be kept in a good condition during the storage. Deterioration, either in transit or in storage, can lead to serious monetary losses. Sugar will not deteriorate if it has the following desired characteristics (5):

- 1) Was boiled from a well clarified juice and is therefore comparatively free from insoluble matter (particles of bagacillo or other insoluble matter hold moisture and serve as breeding places for microorganisms).
- 2) Has a hard, uniform and fair sized grain, free from conglomerates. Large grains have less surface area and will therefore absorb less moisture.
- 3) Has a moisture content in relation to polarization which conforms with certain "factors of safety". The keeping quality is characterized by the safety factor (SF). It is found by dividing moisture % sugar by 100 minus the polarization (non-sucrose) of the sugar. Microorganisms that cause deterioration cannot develop in solutions of high density.
- 4) Was not washed or still surrounded by the original film of molasses.
- 5) Was manufactured under sanitary conditions. Deterioration maybe caused by the action of the following microorganisms in the film of molasses surrounding the crystals: mold, osmophilic yeasts (frequently torulae) and sporogeneous bacteria. These microorganisms attack the sucrose and produce decomposition products as indicated by the fall in polarization.
- 6) Has been cooled. The temperature at the time of dropping is not more than 32°C. Sugar may be kept for extended periods if the following proper storage conditions are satisfied:

#### 1) Good sanitation

Basic requirements of a sanitation program for any food plant must be observed. These includes: those which pertain to personal hygiene and public health of all personnel such as dress, habits and state of health of personnel, sanitary facilities and waste disposal; control measures to prevent contamination by foreign matter (filth) from insects and rodents; and those which pertain to good

housekeeping.

**2) Maintenance of satisfactory humidity and temperature conditions inside the warehouse.**

Raw sugar crystals contain occluded bagasse particles, scale and other insoluble matter. The crystals are always covered by a layer of molasses film which is a major factor in storage problems particularly adhesion (caking) and deterioration.

Sugar will cake under the following favorable conditions:

- (a) mixing of sugars of very different equilibrium relative humidity and of different temperatures,
- (b) varying temperature conditions in different parts of a sugar pile,
- (c) small and irregular grain,
- (d) high moisture content, after the moisture evaporates in part.

The keeping quality of raw sugar is characterized by the safety factor. The control of the moisture content of the sugar by the safety factor is effective for the sugar as produced. However, moisture absorbed during storage is as detrimental as moisture left in the sugar at the centrifugals. The amount of water present in the film of molasses surrounding the crystals is of vital importance. Deterioration will most likely start in this film and will be of a microbiological nature. The rate of microbiological activity is lower at higher concentration of the molasses film. The Equilibrium Relative Humidity (ERH) of a sugar is a measure of its vulnerability to deterioration in storage. The ERH of a sugar is that relative humidity of the atmosphere with which it is in equilibrium or at which the sugar neither absorbs nor losses moisture, at constant temperature. Storage conditions must be met to prevent variations or changes in moisture content. The following are the upper safe limits for temperature and Relative Humidity (8):

- 1. 65% R.H. at 30°C, for sugars polarizing above 97.0.
- 2. 50% R.H. at 40°C, for sugars polarizing above 97.0.

Bulk sugar can be stored satisfactorily in almost any type of warehouse that will keep out the weather and is tight enough to keep air from circulating through the building. To maintain ideal warehouse conditions, the following features are recommended 8, 16, 2 :

- 1. The walls should be strong and reinforced with concrete to a maximum height of 10 feet.
- 2. The floor should be laid on a well consolidated high foundation and should

be water proofed.

3. The roof should be insulated and sloping for efficient drainage.
4. The walls and roof should be painted with aluminum paint on the outside to reflect heat waves.
5. There should be good outside drainage.
6. There must be only a minimum number of windows or other openings. The doors should be of the sliding type.

## WHITE SUGAR

White sugar is either stored in bulk in special silos or in bags or sacks. In the Philippines, sugar is packed as made in 50 kg bags or sacks and stored in warehouses. Some refiners also pack their sugar in 2.5 kg, 2.0 kg and 1.0 kg polyethylene plastic bags. The weighing, filling and sealing of the bags is carried out by automatic packing machines. Proper precautions should be taken in the storage of white sugars. Sugar is properly "conditioned" by the following methods (6) to prevent problems in storage, particularly caking:

1. boil uniform sugar grains, free from conglomerates.
2. remove as much water as practically possible at the centrifugals.
3. dry as slowly as possible at low temperatures.
4. boil and centrifuge sugar at relatively low temperature to minimize residual dissolved sugar in the surface film which will recrystallize during storage.
5. bag sugar at a temperature of less than 38°C.

In order for the sugar to remain in good condition during storage, it must be kept at controlled atmospheric conditions. White sugar always has a thin film of syrup on the surface of the crystals. The syrup is in a highly supersaturated state so that the sugar appears to be dry. However, exposure to an atmosphere of a relative humidity greater than the equilibrium relative humidity of the sugar and fluctuations in ambient temperature would lead to caking. Temperature conditions must remain constant and should not exceed 38°C. The upper safe limit for Relative Humidity is 60%.

The features of a warehouse for ideal storage of raw sugar are the same as that for white sugars. The walls and floor should be of concrete. Pile height should be kept to a practical minimum and if possible, storage should be separate from other products.

Sugar, being a food product must also be a safe product. It must be clear and wholesome. Controls must be taken to guard against harmful outside contamination. The basic requirements of a sanitation program which pertain to personal hygiene and health of personnel; the protection of the public against specific health hazards and also the elimination of potential spoilage hazards must be observed.

## SUGAR MARKETING AND DISTRIBUTION

At present, there are thirty five (35) operating sugar mills in the country with a combined milling capacity of 174,100 metric tons cane per day. The Philippine sugar industry practices the sharing system whereby the planters supply the canes while the millers process or recover sugar from the canes. The two enter a milling contract based on supply of cane; recovery of sugar; payment for manufacturing services and system of sugar distribution. The sugar sharing system is 60 to 70% for the planters and 30 to 40% for the mills. Quedans are issued by the mills for the planters' share of the sugar production. Raw sugar production is divided into four classes, namely: "A" or export sugar; "B" or domestic sugar; "C" or reserve sugar and "D" or world market sugar. Sugar Order No. 2, Series of 1994-95 was issued by the Sugar Regulatory Administration (SRA) 17 last August, 1994. The sugar order authorizes the use of "D" sugar (direct use or for refining) by manufacturers of sugar-based products for export under certain conditions. Each crop year, percentage issuance of quedan on sugar produced is made by SRA to balance sugar production and sugar requirements and also to maintain marketing conditions that will ensure stabilized prices at reasonable levels profitable to producers and fair to consumers. The distribution of the various classes of sugar is based on a preliminary estimate of sugar production and the United States quota for the period. Allowances are made for a deficiency or surplus in sugar inventories and demand conditions.

The marketing of sugar is a free enterprise. The system permits sugar to be bought and sold on a free and open market. It involves distribution of raw and refined sugars mostly by accredited sugar traders. The sugar distribution flow chart is shown in Figure 1.

Raw sugar is produced in Luzon, Eastern Visayas, Panay, Negros and some parts Mindanao. Most of these production areas are a considerable distance from the sugar markets. The sugar traders buy sugar from producers and/or planters and resell to refiners and consumers. Some are also engaged in the exportation of sugar to foreign markets. Traditionally, the United States has been the regular export outlet for Philippine raw sugar. At present, we still export sugar to the United States but under a reduced specified quota. Our importers in the world market include Japan, South Korea, Guam and India.

A large portion of the raw sugar in the domestic market is processed to refined sugar. Part of the remaining sugar enters the industrial market and the rest goes directly to the ultimate consumer market for direct consumption. Refining is done by thirteen (13) integrated (mills with sugar refineries) and three (3) non-integrated refineries. The combined rated capacity is 137,500 L-kg bags per day. The non-integrated refineries purchase raw sugar either from the traders or direct from sugar mills.

The domestic market for refined sugar is a complex market composed of many user groups. The distribution is also done by sugar traders. The traders

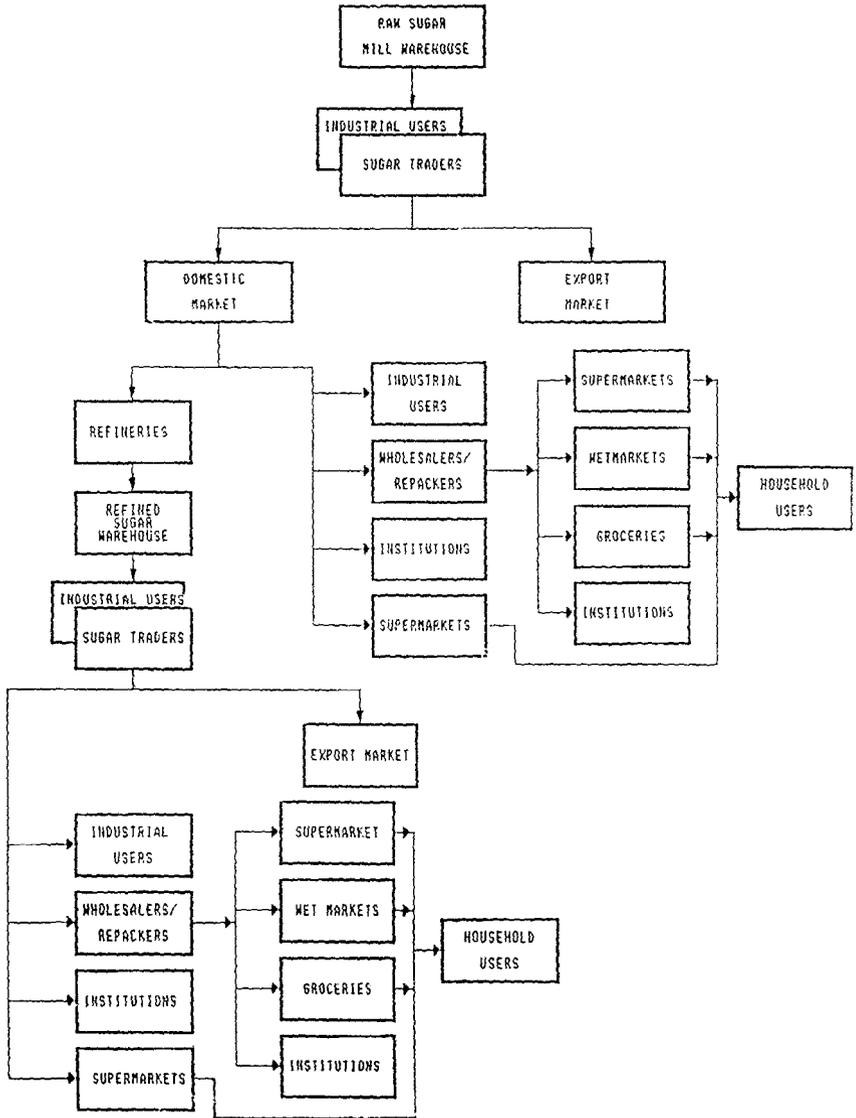
purchase sugar and resell to the consumers through wholesale and retail channels. Industrial users usually buy direct from the refineries through authorized traders since their consumption is substantially larger than the other users. This year, we entered the foreign market for refined sugar by exporting three (3) metric tons of sugar to Guam.

### REFERENCES

1. Annual Synopsis of Production and Performance Data. Philippine Sugar Refineries, Operating Years 1978 to 1993. Factory Operations Department, IR & D, Sugar Regulatory Administration.
2. Baikow, V.E. 1967. Manufacture and Refining of Raw Cane Sugar. Elsevier Publishing Co. pp 205-212.
3. Chen, J. and Chou, C. 1993. Cane Sugar Handbook. Twelfth Edition. John Wiley and Sons, Inc. pp. 48-512.
4. Ibid. pp. 524-532.
5. Ibid. pp. 341-346.
6. Ibid. p. 511.
7. Domino Raw Sugar Contract, 1984.
8. Honig, P. 1963. Principles of Sugar Technology. Vol. III. Elsevier Publishing Co. pp 392-393.
9. Junk, W. Ray and Pancoast, H. 1973. Handbook of Sugars for Processors, Chemists and Technologists. The AVI Publishing Company, Inc. pp. 2-5.
10. Mauch, W. 1980. Quality Factors in Commercial White Granulated Sugar. Vol. 7. Sugar Technology Reviews March. pp 118-133.
11. Philippine Standard No. 061-01; 1976. Philippine Bureau of Standards.
12. Philippine Standard No. \_\_\_; 1980. First Revision. Philippine Bureau of Standards.
13. Philippine Standards No. 061-02; 1976. Philippine Bureau of Standards.
14. Philippine National Standard 1097: 1992. Bureau of Product Standards.
15. Philippine National Standard 1098: 1992. Bureau of Product Standards.

16. Spencer, W. and Meade, G. 1963. Cane Sugar Handbook. Ninth Edition. John Wiley and Sons, Inc. p255.
17. Sugar Order No. 2. Series of 1994-95. Sugar Regulatory Administration

Figure 1. Sugar distribution flow chart.



**Third Plenary Session**  
**FOOD BIOTECHNOLOGY**



# THE APPLICATION OF BIOTECHNOLOGY IN FOOD SECURITY

*Gregory C. Gibbons and Carl J. Gibbons*  
Australian Biotechnological Resources Pty. Ltd.,  
Melbourne, Victoria, Australia

## ABSTRACT

Food security and food preservation are vital to a country's well being. They ensure that the food which is made available to consumers is fresh, nutritious, and in adequate supply. Maintaining food security involves implicating high standards at all ends of the food processing chain, from harvesting or slaughtering, to final packaging and preservation. Problems at different points in the food chain can lead to various difficulties such as food spoilage, low yields of crops or livestock, and often, loss of consumer support. Food biotechnology may be applied at any stage in the food production chain from crop-protection technology to product preservation and packaging. The types of biotechnology which can be applied to ensure food security include both modern genetic technologies, and traditional fermentation methods. This paper discusses the role biotechnology can play in food security, and describes some foods and food additives produced through biotechnology. It is asserted that biotechnology is easily integrated into existing food chain infrastructure, and can optimize food security in the Southeast Asian region.

## FOOD BIOTECHNOLOGY

The topic of biotechnology as it relates to food security and food preservation, covers a very wide range of issues. To determine how food biotechnology fits in to these issues it is necessary to first look at the logistics of the food chain. Table 1 presents a summary of the key items.

To ensure food security, the **infrastructure** of the food chain should be well developed. This means: suitable transport procedures following harvests, suitable transporting vehicles and storage facilities; suitable transfer locations and grading facilities; and a means by which farmers can be rapidly and efficiently paid for their supply of high-quality fresh fruit, vegetables and meat products. If this latter element is missing, may be unwilling or unable to continue to supply their produce.

The **workforce** within the food chain should be adequately trained to understand the sometimes complicated procedural requirements placed upon it to supply fresh produce to markets in a secure form. This can mean on-site training of even the least qualified members of staff, extending all the way to the farm level. The training of quality control personnel at the other end of the food chain is normally not a problem, quality control being acceptance by the consumer of a well-testing, well presented product which is safe to eat and provides nutrition at an affordable price.

**Plant production** applies to the production of fruit and vegetables for the

fresh food market or for subsequent preservation or value addition, as well as production of fodder necessary for animal production (both meat and dairy products). Plant production covers a wide field, some of the key points which can be addressed by biotechnology will be discussed later.

**Animal Production**, to be successful, requires that the breeders and keepers of animals, as well as employees of secondary facilities such as slaughterhouses and packaging plants fully understand the nature of the product they are dealing with and the best means to preserve this.

**Harvesting procedures**, when organized efficiently, will ensure maximum yield from fields of crops, and reduce loss of potential produce by preventing seed damage which can lead to fungal or bacterial infection.

**Slaughtering** of animals can be optimized with efficient pre-slaughter and post-slaughter procedure to ensure high meat quality. Efficient use of by-products from both animal slaughter and plant harvesting procedures can increase on-farm productivity,

**Preservation of raw products**, is vital to the manufacture of a good food product. In southeast Asia, chilling is practiced in the fishing industry, to prevent fresh-caught fish or other seafoods from deterioration. Most plant products and some meat products do not easily give off a strong smell to indicate deterioration, so their freshness and quality is not readily apparent to determine suitability for later processing.

**Food processing** is another important factor in food production. Processing of food by heating, drying or salting has been used in Asia for centuries. More modern technologies such as freezing, canning, or retort processing, have markedly increased shelf-life and added value to raw agricultural commodities. Traditionally used in Asia as a preservation technique is fermentation. The resulting lower pH of the food precludes harmful fungi or bacteria resulting in a product which requires little or no refrigeration.

Food chain logistics have changed greatly in the last century due to increasing urbanisation. In the past, fresh plant or animal produce was only harvested or slaughtered immediately prior to consumption, and thus food preservation was not required. Wastage was also minimal. Urbanization has removed areas of production away from areas of consumption. Consumers therefore became dependent on market systems, which would collect food from primary producers, store it and consolidate it to required mix prior to delivery to the consumers. With the rapid expansion of the electricity net within southeast Asia during the 70's and 80's, refrigeration technology became important to the whole food chain. Today, many supermarkets contain preserved food products which can only stay fresh in the presence of electrical refrigeration.

### **The Role of Biotechnology in Food Security**

Biotechnology assists a wide area of food technologies, both with modern genetic techniques, and traditional microbial applications such as fermentation.

## 1. Plant Breeding

Biotechnology has provided a number of very useful tools to the plant grower. These tools make possible rapid test for strains of plants suited to a particular environment, and identifications of high yield strains. Plant breeding biotechnology has also made possible combination of genes from dissimilar species, thus adding specific characteristics to a chosen plant species which would not have been easily attainable using traditional breeding techniques.

## 2. Animal Breeding

Biotechnology in the field of animal breeding has focused on in vitro fertilization and cloning of high-quality animal breeding stocks. Gestation time the breeding potential of the herd. Using embryo sexing and subsequent cloning technologies, it is possible to increase the number of females in the herd, thus decreasing the time it takes to develop new herds of better-quality animals. It is also now possible, with biotechnological techniques, to change the genetic make-up of animals, increasing of growth rate. Transgenic pigs, which contain extra gene bases for the production of porcine growth hormone, have been developed; these pigs develop at markedly faster rates.

## 3. Plant Health

Plant growth is limited by area, sunlight, soil nutrient levels, and available water. Even though farmers provide plants with the water, sunlight, area and nutrients they need, many crops can be lost to plant diseases, pests (esp. insects) and other debilitating afflictions. The use of pesticides and fungicides has become very common as an economic means to decrease the damage plant disease causes. The cost of these fungicides and pesticides is high, and places a great strain on the economy of many developing countries. The environmental costs of widespread pesticide and fungicide use are also of great concern, because the breakdown compounds of many of these chemicals are harmful to animals and humans.

Biotechnology has now provided means rapid diagnosis of plant diseases. Specific targeted use of chemical agents against plant diseases thus diagnosed can decrease the overall use of chemicals, due to the fact that the chemicals are used on a need-only basis. Biotechnology also provides the possibility of incorporating genes into plants which give resistance to disease or pests. The incorporation of *Bacillus thuringiensis* genes in plants has recently been achieved, and offers a great step forward in protection of plants against insects, because the genetically engineered plants use their own internal mechanisms to defend themselves against certain pests rather than needing chemical protection.

#### **4. Animal Health**

Losses in animal production due to pests and microbial infections are high, and in Asia, there are a number of debilitating animal diseases which are endemic in herds which result in an overall lowering of production by 30 to 40 percent. The development of animal health diagnostics and vaccines using biotechnology, has provided an answer to many of the most pressing animal health problems.

#### **5. Plant Senescence Control**

The aging of plants and plant products is a natural phenomenon. This aging, or senescence, often results in fruits and vegetables becoming inedible before they reach the market, due to long transport times lack of facilities for refrigeration. Biotechnology is providing us with an opportunity to incorporate genes in plants which slow down senescence at normal room temperature, eliminating the need for refrigeration. Such technology will unquestionably increase yields of plant produce in the future.

#### **6. Microbial Control**

Often, a lack of microbial control in plant and animal foods leads to gross spoilage of food stocks. Efficient microbial control is now possible using rapid test kits developed through biotechnology, which can replace the time consuming plating procedures used in the past.

#### **7. Fermentation of Additives**

Biotechnology can be used to produce a number of food additives which increase the value and quality of processed foods. These additives will be described in some detail later.

#### **8. Fermentative preservation**

Preservation of products via fermentation, apart from traditional fermented foods as described above, has been used for centuries in animal fodder production. The procedures have relied on the natural inoculation of fresh plant material with yeast and bacteria from the air.

#### **9. Fermentative Value Addition**

Biotechnology has a particular role in value-addition via fermentation. With the correct choice of microorganisms, it is possible to change the nature of the substrate material to a more nutritious and commercially valuable product. Some aspects of this will also be discussed later.

## 10. Diagnostic

Biotechnology has a particular role in the field of diagnostics for use in food security applications. Not only can such diagnostics be used in domestic food processing, they can also be used on imported foodstuffs. These diagnostics kits can be operated by minimally-trained staff, and ensure the security of food entering a country. They can also be used to detect adulteration in foods.

## 11. Waste Treatment

Waste treatment is carried out with some form of biotechnology. Aerobic or anaerobic breakdown of agricultural wastes, food processing wastes, or even human and animal wastes all involve biotechnology.

## TRADITIONAL FERMENTED FOODS

In the Asian region, many foods have been fermented as a means for preservation for ages. A summary of the major traditional fermented food is shown in Table 2. This table is by no means complete, but it attempts to provide an overview of the wide range of products which have been preserved using the traditional biotechnological technique of fermentation.

**Fermented seed-cake** is commonly found in the southeast Asian region. Three examples from Indonesia have been given: those from peanut, soybean and coconut. Often the plant substrate used for the fermentation process is a secondary product of another form of food processing. A good example of this is *tempe*. Following production of bean curd - which is achieved by boiling soy bean - the residue is inoculated with a culture of *Rhizopus* spp., and packed in banana leaves. This is stored at room temperature (around 27-28°C) until the seed-cake is covered with a white mycelium. This product is then cut into slices and fried. It has been traditionally used as a protein source in the Indonesian diet, and produced by more than 80,000 producers throughout the country, mostly family operations.

**Cereal seed porridges** is popular in Africa after cooling porridge is allowed to undergo rapid fermentation, to bring the alcohol content to about 2.5%. These are consumed within 36 hours of manufacture. It is interesting to note that in Botswana, the traditional *chibuku* is now produced in large factories, and supplied daily in large packed containers. The *chibuku* breweries include full quality assurance and quality control laboratories, and the food, though traditional, is produced to the highest standards.

**Sugar solutions** have also been fermented for generations. Of particular note are *basi* from the Philippines, and *mead* from Scandinavian, which is made from honey, and is attributed to have given the Vikings their great strength! Preservation of the rich fish resources of the Asian region has occurred in the past in

many ways. Sun-drying and salting have been to some extent replaced today by canning and freezing.

**Fermented fish products** such as nam pla and kapi from Thailand, budu from Malaysia, and patis and bagoong from the Philippines, are well-known examples of these. These products are normally used as sauces or pastes, and have mild aroma while a fermented herring products from Sweden called sur stroemming, has such a strong aroma that it can only be eaten outdoors.

One of the most ubiquitous fermented foods in southeast Asia today are **soy bean sauce** and **soy bean vinegar**. These foods are produced in thousands of small factories throughout the region, using a combination of surface and submerged fermentation processes.

**Fermented cereal grains** such as tape, are popular in Asia. Tape is fermented rice product slightly alcoholic often enjoyed as a sweetmeat due to its sugary taste and pleasant consistency.

**Vegetables** preserved by fermentation, such as kim chi in Korea, or sauerkraut in Germany, have long been appreciated by local consumers. The mildly acidic crisp vegetables provide an excellent complement to meat dishes.

**Fermented soy bean-extracts** such as tofu or the well-known miso from Japan are good examples of fermented products which have wide application in the region. Fermented garri such as taoco is also done in Indonesia.

**Leavened bread** A traditional fermented food from Northern Africa and Europe, is a relatively recent development in the Asian region. Fermentation procedures prior to baking are divided into two main areas. The yeast fermentation (normally seen in French breads) provides large holes in the bread and a soft, compressible crump texture. The northern European rye breads are utilize bacterial fermentation, resulting in a much more solid and compact bread with smaller air holes. It also has a slightly acidic taste, resulting from the bacterial fermentation.

**Beer** derived from barley malt had its origins thousands of years ago in Egypt. Technologies became highly developed in Europe over the last 400 years, and some of these technologies are currently being developed and successfully used by brewers in Asia today. Beers made from wheat malt have been well-accepted in Europe for many years, and in Africa and the southern areas of the US, beer is made with sorghum malt.

**Mushrooms** are products of the biotechnology. The black mushroom, the abalone mushroom, or the straw mushroom are the most well known. The white button mushroom has recently been introduced from Europe. This mushroom does not thrive in hot temperatures, but biotechnological work has been carried out here in the Philippines by the Industrial Technology Development Institute to increase the temperature-tolerance of the European mushroom.

### NEWER FERMENTED FOODS

Some of the newer fermented foods are shown in Table 3.

**Cheese** is a newer product of biotechnology. Fresh milk is treated with an enzyme originally isolated from the gut of young calves, known as rennin (or chymosin C). Now by modern biotechnology this enzyme is produced by genetic engineering instead of being extracted from calves gut. Rennin coagulates milk, which is subsequently allowed to settle, forming the cheddar. The liquid buttermilk runs off, and the product is pressed and salted prior to storage. While the production of some cheeses does not normally involve fermentation, the production of other cheeses such as blue-vein, camembert, brie, or stilton, requires the cheeses to be fermented and “aged” using a fungal inoculum.

There are a number of new fermented milk drinks in Europe, and some of these are slowly finding their way into Asian markets. Many of these, however, require refrigeration to be kept fresh. The products often contain artificial or natural flavouring. Yoghurt products, as well as sour cream are becoming more popular in Asia, following the increased availability of refrigeration in the region.

## FOOD INGREDIENTS USING BIOTECHNOLOGY

Biotechnology is today used to produce a number of food ingredients. Some of the main food ingredients produced using biotechnology are presented in Table 4.

**Amino acids** are probably the most well-known products of biotechnology to be used as additives. Glutamic acid, used in monosodium, glutamate, has been a key element in Chinese cuisine for many years. It is produced by simple fermentation processes. Lysine, an amino acid which often lacking in animal products, is now also produced in Asia using controlled fermentation.

**Glucose** can be produced by the enzymic action of amylolytic enzymes such as alpha amylase and amyloglucosidase (also known as glucoamylase) on starch. Starch is commonly extracted from cassava, sago, wheat, and other plants. Enzymic activity on starch can also lead to production of high fructose syrup. The technology for high fructose syrup production has been developed using maize starch as the raw material.

**Modified starches and maltodextrin**, used widely in flavouring and modifying texture in food stuffs, have also been produced by enzymic process, sometimes combined with chemical action on starch. **Sorbitol**, a nonsugar sweetener commonly used in toothpaste and candies, is produced by enzymic hydrolysis of starch, followed by chemical hydrogenation of the glucose produced. There are a number of sorbitol-producing facilities in the Asian region.

**Aspartame**, the dipeptide sweeter, is now widely used in the “Diet” drinks, foods and candies. Aspartame can be produced by direct chemical synthesis, or using enzymes to join one molecule of aspartic acid to one molecule of phenylalanine, using reverse peptide cleaving technology.

Flavour enhancers are often produced from combinations of **nucleotides**. These nucleotides are formed by hydrolysis of nucleic acids normally extracted from rapidly growing microorganisms.

**Citric Acid** is widely used in soft drinks and in confectionery. Citric acid production facilities can either use submerged fermentation or surfacer fermentation.

Proteolytic enzymes are widely used in washing powders to remove oily or proteinaceous stains of and in the tenderizing of meat. Of particular note from the Asian countries are bromelain from pineapple, and papain from papaya.

Starch-breakdown enzymes, or **amylolytic enzymes**, are widely used as ingredients in processed foods of the Asian region. In particular, these enzymes are used in the production of ingredients as described above (e.g., high fructose syrup, sorbitol, maltodextrins, etc.) but they are also used in the production of many bread types, to break down starch rapidly, thus allowing the yeast to ferment simple sugars and produce carbon dioxide, which flavours the bread.

### **The Future Role of Biotechnology in Asia**

Biotechnology has a guaranteed future role in the assurance of food security and enhancement of food sufficiency in Asia. The areas in which biotechnology will play a key role are noted in Table 5.

Biotechnology is a means to improve plant crops for the region: with better resistance to pests and diseases, and increased yields. Improved breeding in farm animals, will increase the food conversion efficiency animals, enabling them to use plant resources fully. Similarly, biotechnology can decrease losses from both plant and animal diseases by incorporating pest and disease resistance genes, as well as providing improved diagnosis techniques, and better animal vaccines.

The use of biotechnology in manipulating the post-harvest characteristics of fruits and vegetable will be important to increasing the efficiency of sufficiency of food production in the region. Anecdotal evidence from the Philippines indicates that approximately one half of the tropical fruit harvested is damaged irreparably between harvesting and final supply. Increasing use of biotechnologically-based post-harvest technology will allow for efficient production without added inputs. Improved diagnostic tools developed by biotechnologists will be used to reduce spoilage during processing and storage of foods. A better understanding of relationships between plants, microbes and the soil, will lead to improved efficiencies of plants in the Asian region. Pioneering work in this respect has been carried out by Biotech in Los Banos, here in the Philippines, where commercial preparations of ectomycorrhizal fungi are now produced and sold to farmers.

Another area in which biotechnology can advance food security in the Asian region is the development of materials for storage and packaging for increased shelf life of examples are molecular scavenging compounds.

Finally a key area where biotechnology will assist food security in Asia is

in the improved supply and quality of traditional foods. Simple quality control and quality assurance techniques are being developed based on modern biotechnological diagnostic methods, and introduction of these into the area will assist in assuring safe foods. To assist this development, biotechnology be used to produce additives at an affordable price which can supplement limiting nutritional factors of existing foods in each country, thus increasing use of that country's won food reserves.

## CONCLUSIONS

Food biotechnology will play a major role in the development of food security and food sufficiency in Asia for the future. In this plenary paper, we have attempted to give a broad overview of the scope of food biotechnology as it is today, and to indicate areas in which food biotechnology will play an important role in the future, here in the Asian region. The effective use of current and new food biotechnologies by regional food producers will rely heavily on the development of human resources capable of fully utilizing the technologies. The practical use of these new technologies will subsequently lead to regional adaptation and innovation based on a thorough understanding of the principles and practice involved. To achieve this end, it is imperative that regional networks be expanded to ensure the best possible use of the currently limiting human resources in food biotechnology in Asia.



**Session 4**  
**Food Ingredients and**  
**Additives**



# PRODUCTION OF LACTIC ACID BACTERIA FOR FOOD INOCULANT AND PRESERVATION

*Laura J. Pham, Chay B. Pham and Dominic D. Villa*  
National Institutes of Biotechnology and Applied  
Microbiology (BIOTECH), U.P. at Los Banos, College,  
Laguna 4031, Philippines

## ABSTRACT

Lactic acid bacteria are often used as inoculants and preservation agents in the manufacture of food, pharmaceutical preparations and chemical feedstocks. The major problems in the cultivation of lactic acid bacteria are product inhibition, less substrate consumption, low growth rate and expensive culture medium. A cultivation process of high cell concentration was studied to develop a low cost medium and increase the specific growth rate, substrate consumption and product formation.

The effect of various sugars used in the culture medium were optimized using *Lactobacillus acidophilus* in batch and continuous process. The highest specific growth rate (0.579 h<sup>-1</sup>) was obtained with lactose in whey based medium. A concentrated suspension of bacteria cells were performed in a continuous process using a stirred tank fermentor coupled with a ceramic filtration system under non-aerobic conditions. A productivity of  $3.25 \times 10^{10}$  cells cm<sup>-3</sup>h<sup>-1</sup> was achieved and a 10.85 fold increase in productivity was seen as compared to the batch fermentation. A 90%(w/w) substrate in the culture medium was consumed and the product has no inhibition effect in the simultaneous system of continuous feeding and filtration process.

## INTRODUCTION

Fruit and vegetable processing eliminates postharvest wastage and spoilage, provides better returns to growers, and assures consumers of supply all-year round, thus playing a major role in the attainment of food self-sufficiency.

This paper will attempt to present an updated situationer on the fruit and vegetable processing industry in the rural areas in the Philippines, data on exports of processed fruit and vegetable products, technologies developed at the Bureau of Plant Industry and their possible application to village-type processing plants, and advantages of putting up village-type processing plants.

## THE FRUIT AND VEGETABLE PROCESSING INDUSTRY IN THE RURAL AREAS

In the Philippines, the fruit and vegetable processing industry is characterized by the dominance of cottage and small scale traditional establishments located mostly in the rural areas. In these establishments processing operations

differ in their level of technology and degree of sophistication. They are often family-owned, staffed mostly with women workers.

By 1988, the registered food manufacturing establishments totalled 33,429. Classified based on employment size, 93.73% or 31,335 belong to the cottage level with less than 10 employees. The rest of the industry constitutes the following: 1,864 or 5.58% for the small-scale, with 10-99 workers; 83 or 0.25% for the medium, with 100-199 workers; and 147 or 0.44% for the large, with 200 or more number of workers. The industry is described as those establishments engaged in canning and preserving fruits and vegetables; fruit and vegetable juices; fruit and vegetable sauces; peanut and peanut products; cassava/arrowroot flour, starch and its products.

### PROCESSING EQUIPMENT

A wide range of processing equipment can be observed since little capital is invested in machinery. Some entrepreneurs improvise with rudimentary equipment and some are second-hand or locally-fabricated. Among the simple processing equipment commonly found are gas-fired cooking kettles, roasters, mechanical grinders, blowers, cutters, drying trays, ovens, and aluminum tables. There are manufacturer/exporter firms who do not invest in machinery but prefer to rent for the use of facilities. However, other successful manufacturer/exporter firms invest in machinery like the Lubag Food Corporation located in Morong, Rizal, which manufactures assorted heat-processed products like nata de coco, coco juice, kaong, fruit cocktail, pickled vegetables, tomato sauce, sweet corn, purple yam jam and novelty products like heart of palm, banana blossoms and green jackfruit. Installed in its plant are equipment like can seamer, retort, slicer (mobile), steam jacketed kettles and exhaust box. For installation are equipment obtained through a government financing loan like dehydrator, filling machine, pulper, crusher, and boiler. Lubag Food Corporation is a family-owned firm which employs eight (8) regular staff and hires six to ten persons when there are product orders mostly from Japan, Korea, Taiwan and the European countries.

In Lobo, Batangas, a very small town located 116 kms. away from the metropolis, candied/glazed tamarind is being processed still in the crude way. Cooking is done in large cast-iron vats using firewood as fuel. The cooked fruits are drained, arranged in drying trays and sun-dried atop the building with galvanized roofing. The dried fruits are packed in wax-lined boxes and covered with cellophane. A wholesaler takes charge of the marketing and distribution in groceries and supermarkets almost nationwide. Another example of a simple rural processing technology is starch processing from arrowroot. Arrowroot (*Maranta arundinacea* Linn.) is one of the lesser known rootcrops grown wildly in many parts of the country. The fine, highly digestible starch from the rhizomes has reportedly many uses but in the Philippines, the existing processing is mainly the making of starch for special biscuits called "uraro". Results of a survey disclosed that arrowroot farmers are at the same time processors, each farmer maintaining his own processing unit consisting of a makeshift shed which houses the

presser and sedimentation containers.

The rhizomes are washed, peeled and re-washed to remove dirt and bitter taste. Crushing is done through the use of an improvised presser made of cement (approximately 4 feet long and 1 foot wide) molded around a 6 foot cylindrical log. It serves as the weight needed to crush the arrowroot. The protruding log ends rest against two thick slabs of timber supported by solid posts. The crushed pulp is mixed with lots of clean water, passed through a series of sieve to separate the fiber and allowed to settle in sedimentation containers, e.g. basins or cemented tanks. Water is drained from the settled starch and sun-dried until lumps are thoroughly pulverized. The starch is then packed in moisture-proof bags.

### **Packaging**

Batch type processing and packaging is employed with minimum sophistication. The use of plastic is the most common type of packaging material. For heat-processed products, bottling is widely practiced, using recycled bottles with new caps.

### **Marketing**

These products have varying outlets although many are sold in groceries and supermarkets. Some primary processed foods are sold to large food processors as ingredients for their operations, e.g. fruit purees to ice cream and to fruit juice manufacturers. There are also cottage and small scale types which export processed fruit and vegetable products together with the large ones.

Statistics compiled by the Bureau of Export Trade Promotion show that in 1992, there were 304 exporters of processed fruits and vegetables (Table II). Majority of these exporters are located in the National Capital Region or in the urban centers. However, the three companies based in Northern Mindanao have the biggest impact on regional investment and employment (12).

## **EXPORTS OF PROCESSED FRUIT AND VEGETABLE PRODUCTS**

Processed fruits and vegetables in various forms remain to be among the country's regular and promising exports. Table III shows the quantity and value of the different processed fruit and vegetable exports for a 3-year period, from 1991-1993. There was a 32.19% increase in the quantity of exports from 1991 (259,707.6 kg) to 1992 (383,048.9 kg) and a 21.04% decline from 1992 (383,048.9 kg) to 1993 (316,469.1 kg). Also shown in descending order were the commodities exported in 1993, as follows: (1) fruits in syrup which tops the list; (2) dried rootcrops; (3) juices, concentrates, purees, and pastes; (4) other food preparations which include banana chips/crackers, banana ketchup and flour, flour meal and powder of fruit and nuts; (5) food preserves like beans, nata de coco, macapuno, kaong; fruits and vegetables in vinegar/acetic acid; (6) dried fruits; (7) jams, jellies and marmalades;

(8) nuts (peanut); (9) dried vegetables and legumes; and (10) fruits in brine/frozen.

From January to November 1993, the country earned \$15.5 million from nata exports. This figure was fifteen times the export for 1992. Japan accounted for 94.3 per cent while smaller shipments went to the United States, Taiwan, Australia, Hongkong, Saudi Arabia, Canada and the Netherlands.

The United States is the major foreign market for pineapple syrup, concentrates and juices. There is also a growing trend in the exportation of dried fruits. In the United Kingdom, Germany and France, bananas and papayas are the most popular among the tropical dried fruits, used as snack food and as ingredients in breakfast cereals, ready-to-eat meals and desserts (7).

### TECHNOLOGIES AVAILABLE ON PROCESSED FRUITS AND VEGETABLES

Despite its simple facilities and equipment, the Laboratory Services Division of the Bureau of Plant Industry continuously develops processing technologies for indigenous fruits and vegetables.

Listed in Table 2 are selected crops with their corresponding scientific name and developed technologies which have possible application for village level processing. As indicated, simple and less costly processing techniques have been developed on the following food products: candy, jelly, jam, pickle, nectar, powder, sauce, chips, crackers, wine, catsup or simply dried and canned in syrup.

1. Candied banana, catsup, flour and wine have been developed from Banana, *Musa sapientum* var. *Cavendish*
2. Chips/crackers and flour developed from Banana, *Musa sapientum* var. *Compressa*
3. Juice, syrup, candied rind from Philippine lemon "kalamansi", *Citrus Microcarpa Bunge*
4. Jelly, nectar, and wine from guava (*Psidium guajava* Linn.)
5. Nectar, jam and frozen form from Soursop (*Anona muricata* Linn)
6. Candied, canned in syrup from jackfruit (*Artocarpus heterophyllus* Lamk)
7. Canned in syrup, jam, pickled, dried, puree, nectar and leather from mango (*Mangifera indica* Linn.)
8. Nectar, jelly, jam, canned in syrup, candied, dried and pickled products from papaya (*Carica papaya* Linn.)

9. Jelly, jam, syrup from passion fruit (*Passiflora foetida* Linn)
10. Canned in syrup, jam, candied, dried, frozen, wine from pineapple (*Ananas comosus* Linn.) Merr.
11. Canned in syrup from rambutan (*Nephelium lappaceum* Linn.)
12. Canned in syrup, preserve, candied, wine from santol (*Sandoricum koetjape* Burm. F.) Merr.
13. Flour and starch from Cassava (*Manihot esculenta* Crantz)
14. Powder from purple yam (*Dioscorea alata* Linn.)
15. Instant tea, dried product from ginger rhizome (*Zingiber officianale* Rosc.)
16. Soysauce, tokwa, tau-hu from soybeans (*Glycine soja* L. Sieb. and Zucc.)
17. Catsup, sauce, pickled, wine from tomato, *Lycopersicon lycopersicum* L. (Karst)

In addition, the Bureau has also developed its own technology for nata de coco production and processing from both coconut water and coconut milk.

Production and processing of these products could be done using simple equipment in rural households although economies of scale in production is achieved through the use of modern sophisticated equipment.

One of the mandates of the Bureau is to transfer these matured technologies to interested persons in support of the national livelihood program. This is achieved through the conduct of regular seminar-workshops and lecture-demonstrations. Judging by the list of participants, the technology transfer project seems to attract and benefit households, retirees and would-be entrepreneurs.

#### **ADVANTAGES OF ESTABLISHING VILLAGE-TYPE PROCESSING PLANTS**

Different sectors in the rural areas abound with seasonal crops which generally find its way to local markets for consumption in the fresh state. At the peak of production, surplus go to waste with inevitable losses. Establishment of village level processing plants which could be integrated with the production activities of the area would minimize postharvest losses. Several positive factors could also be generated like employment opportunities for the rural population, thus preventing migration from rural to urban areas. This would also result to increased farm income, improved diet, help overcome seasonal gluts and short-

ages of produce, thus contributing to overall integrated rural development.

To further stress the point, let me quote from a FAO Paper on Enhancing Rural Employment and Incomes Through the Development of Agro-Processing Industries. "Dispersal of rural cottage industries and simple processing plants throughout the countryside side, close to the supply of raw material, drawing labor from the farming community, increases linkage between agriculture and industry".

Needless to say, food processing plants could only be established in areas with adequate potable water and electricity. Energization of rural areas is continuously being done. As of May 1994, the National Electrification Administration reported that out of 41,910 barangays (COMELEC -May 1994 elections) 23,153 barangays had been energized. On the other hand, the Local Water Utilities' Administration (LWUA) reported that 583 water districts had been formed nationwide as of December 1993.

#### REFERENCES:

1. Arrowroot Culture and Processing. 1987. Produced by the Laguna Countryside Action Project and Communication Staff. Dean's Office, College of Agriculture.
2. Bureau of Export Trade Promotion. EDP
3. Commission on Elections. Statistics Department.
4. Crisostomo, Lydia C. "Fruit and Vegetable Processing in the Rural Areas". Paper presented during the Agri-Aqua Fair held at Philcote on May 30, 1988.
5. "Enhancing Rural Employment and Income Through the Development of Agro-Processing Industries". 10-14 February 1992. FAO Paper. Twenty-First Regional Conference for Asia and the Pacific. New Delhi, India.
6. Food Composition Tables Recommended for Use in the Philippines. 1990. Food and Nutrition Research Institute. Department of Science and Technology. Manila, Philippines.
7. Harman, G. and N. Anand. 1990. The Market for Dried Fruit in the United Kingdom, The Federal Republic of Germany and France. Overseas Development Natural Resources Institute Bulletin No. 34, vi + 70 pp.
8. Local Water Utilities Administration. 1993 Annual Report.
9. Lustre, Alicia O. and Caridad F. Aspiras. 1981. "Status of the Food Pro-

- cessing Sector". 1981. Food Map of the Philippines and How to Establish a Food Processing Business. Productivity and Development Center. DAP.
10. National Electrification Administration. Planning Department.
  11. Preservation of Fruits and Vegetables. 1983. Laboratory Services Division, Bureau of Plant Industry, Department of Agriculture, Manila, Philippines.
  12. Processed Food Industry: A Turf of SMEs. 1994, September 18. The Philippine Star.
  13. Roa, Julieta. "Profile of Arrowroot Farming and Processing". Paper presented for the Seminar-Workshop on R & D Status of Promising Rootcrops, PCARRD, Los Banos, Laguna, October 11, 1989.
  14. SME Statistical Report. June 1992. Bureau of Small and Medium Business Development, Department of Trade and Industry, Manila Philippines.

**Table 1. Quantity and value of processed fruits and vegetables exports (1991-1993)**

COMMODITY	1993		1992		1991	
	Quantity (kg)	Value (\$)	Quantity (kg)	Value (\$)	Quantity (kg)	Value (\$)
Fruits in syrup	131,928,632	65,155,985	212,142,310	105,642,621	208,041,490	103,868,617
Dried rootcrops	116,018,630	2,702,592	58,574,063	8,344,657	38,888,427	5,341,735
Juices, concentrates, purees pastes	59,842,302	25,723,485	93,570,877	77,743,385	97,833,944	52,008,461
Other food preparation (chips/crackers; banana ketchup and flour; flour meal and powder of fruit and nuts)	7,102,091	6,636,230	12,241,473	13,867,759	13,652,619	15,500,979
Food preserves (beans, nata de coco, macapuno, kaong)	655,529	1,410,959	1,679,301	2,977,647	1,200,776	2,290,784
Fruits and vegetables in vinegar/acetic acid/frozen not frozen/salted and fermented	417,551	759,325	1,201,841	1,572,060	988,284	1,311,987
Dried Fruits	273,422	998,491	1,126,028	5,050,839	1,107,802	4,803,229
Jams, jellies and marmalades	149,478	293,227	1,762,329	1,559,661	1,157,759	1,040,998
Nuts (peanut)	62,399	190,341	268,148	608,298	288,627	664,211
Dried vegetables & legumes	15,261	20,365	123,798	111,134	408,610	103,102
Fruits in brine and frozen	3,768	12,136	358,811	1,081,435	312,146	925,657
<b>Total</b>	<b>316,469,063</b>	<b>183,983,136</b>	<b>383,048,979</b>	<b>218,558,696</b>	<b>259,707,605</b>	<b>187,850,760</b>

SOURCE: Central Bank of the Philippines (Bangko Sentral ng Pilipinas)

**Table 2.** Developed technologies from selected fruits, vegetables and rootcrops.

Crop (English Name)	Scientific Name	Technologies (Processed Products)
<b>Fruits</b>		
1. Banana	<u>Musa sapientum</u> var. Cavendish	Candied, catsup, flour, wine
2. Banana	<u>Musa sapientum</u> Linn. var. <u>Compressa</u>	Chips/crackers, flour
3. Phil. Lemon (Kalamansi)	<u>Citrus microcarpa</u> Bunge	Juice, syrup, candied rind
4. Guava	<u>Psidium quajava</u> Linn	Jelly, nectar, wine
5. Soursop	<u>Annona muricata</u> Linn	Nectar, jam, frozen
6. Jackfruit	<u>Artocarpus hetero-</u> <u>phyluss</u> Lamk.	Candied, canned in syrup
7. Mango	<u>Mangifera indica</u> Linn.	Canned in syrup, jam, pickled, dried, puree, nectar, leather
8. Papaya	<u>Carica papaya</u> Linn.	Nectar, jelly, jam, canned in syrup, candied, dried, pickled
9. Passion fruit	<u>Passiflora foetida</u> Linn.	Jelly, jam, syrup
10. Pineapple	<u>Ananas comosus</u> (Linn.) Merr.	Canned in syrup, jam, candied, dried, wine frozen
11. Rambutan	<u>Nephelium lappaceum</u> Linn.	Canned in syrup
12. Santol	<u>Sandoricum koetjape</u> (Burm. F.) Merr.	Canned in syrup/preserve candied, wine
13. Cassava	<u>Manihot esculenta</u> Crantz	Flour, starch
14. Yam (purple)	<u>Dioscorea alata</u> Linn.	Powder
15. Ginger rhizome	<u>Zingiber officinale</u> Rosc.	Instant ginger tea, dried
16. Soybean	<u>Glycine soja</u> L.Sieb. & Zucc.	Soysauce, tokwa, tahu
17. Tomato	<u>Lycopersicon lyco-</u> <u>persicum</u> L. (Karst)	Catsup, sauce, pickled, wine



# EASTMAN SAIB-SG FOR BEVERAGE APPLICATIONS

*Lee Lai See*

*Eastman Chemical Limited, Singapore*

## ABSTRACT

Sucrose acetate isobutyrate, special grade (SAIB-SG) is widely used as a weighting agent in citrus-flavored beverages. SAIB-SG is currently permitted under the food laws of more than 20 countries.

Eastman SAIB-SG is an excellent weighting agent for flavor emulsions used in both carbonated and noncarbonated citrus-flavored beverages. It is produced by the controlled esterification of natural sugar (sucrose) with acetic and isobutyric anhydrides.

Eastman SAIB-SG has many advantages over other currently used beverages weighting agents, such as brominated vegetable oil (BVO) and glyceryl esters of wood rosin (ester gum).

SAIB-SG is flavorless at typical beverage levels and is readily soluble in flavoring oils, vegetable oils and animal fats. Eastman SAIB-SG has undergone extensive safety testing on laboratory animals and humans. SAIB-SG is metabolized to sugar (sucrose), acetic acid and isobutyric acid, all which are safe and common components of foods.

While traveling around the globe we see a wide variation in people's food and drink preferences, but one type of product you can find almost anywhere are citrus flavored beverages. Orange, lime and lemon flavors are very popular, but certainly not the choices available. This presentation will focus on how sucrose acetate isobutyrate special grade (SAIB-SG) can improve the stability of cloudy citrus beverages.

If you look at the ingredients used in cloudy citrus beverages you are most likely to see the following: water the universal diluent, a sweetener, an emulsifier either Acacia Gum or modified starch, an acidulant possibly citric acid, a preservative, and of course a source of citrus flavoring. But, the ingredients used to flavor these beverages may differ around the world.

The flavorings used can be either whole fruit juices or essential flavoring oils, or a combination of juice and essential oil. If whole juice is used some of the natural compounds in the juice will help stabilize the finished beverage. This is an advantage of whole juice, but there are several potential disadvantages, such as storage tank limitations, possible spoilage, and year to year variability in the fruit crop. If essential flavoring oils are used as the flavoring compound, density adjusting agents, sometimes called weighting agent, are often used to stabilize the finished beverages.

Essential flavoring oil are concentrated citrus flavors. They contain unsaturated oils which have a low solubility in water, and a specific gravity less than water. Density adjusting agents are lipophilic compounds, oil soluble, with a specific gravity greater than 1. If essential flavoring oils were used without a density agents they would have a tendency to separate from the beverage leaving a ring of oil on the top of the beverage. Stokes Law of Sedimentation can be used

to examine why this separation or ringing occurs in a beverage. Stokes Law states

**Stokes Law of Sedimentation:**

$$v = \frac{2 \times g \times r^2 (d_1 - d_2)}{g N}$$

$v$  = Velocity of sedimentation     $g$  = gravitational constant

$N$  = viscosity     $r$  = radius of droplets

$d_1$  = density of oil phase     $d_2$  = density of aqueous phase

Since  $g$  is a constant and the viscosity of beverage can not be altered significantly the factors we can change to effect beverage stability are the radii of the oil droplets, and the density difference between the oil phase and the aqueous phase. Oil droplet radii should be less than 1 micron for the best beverage stability. Since this term of Stoke's equation is squared special attention on mixing and homogenizing is important. This leads to the main subject of this presentation, adjusting the density difference between oil phase and aqueous phase to increase the stability of the finished beverages.

Density adjusting agents are used to increase the specific gravity of the flavoring oil phase to more closely match that of the aqueous phase. The specific gravity of an orange flavored essential oil is approximately 0.84, while a nondietic beverage has a specific gravity close to 1.05. Density adjusting agents like sucrose acetate isobutyrate special grade, are used to decrease this specific gravity difference.

The three most common density adjusting agents used today are sucrose acetate isobutyrate special grade, brominated vegetable oil, and glyceryl esters of wood rosin. I will briefly discuss each of these density adjusting agents, and then explain how sucrose acetate isobutyrate special grade can be used in beverage formulation.

Brominated vegetable oils (BVO) have been used in beverages for many years. Some advantages of BVO include: it has a high specific gravity, approximately 1.33 if soybean oil is used as a starting material, and it provides a highly dense cloud to the finished beverage. The disadvantage of BVO is the perceived health risk of using brominated compounds in food products. BVOs are either restricted to very low levels or banned in many countries around the world.

Glyceryl esters of wood rosins, sometimes called ester gums or glyceryl abietate have also been accepted for many years in certain parts of the world. Some advantages of glyceryl esters of wood rosin include: they are solid and produce very stable cloudy emulsions. The disadvantages include: a distinctive rosin like taste, they do not readily dissolve in essential oils, they are oxidate unstable, and it is difficult to clean equipment after using glycerol esters of wood rosin.

Sucrose acetate isobutyrate special grade is the most recent density adjust-

ing agent used in beverages. It is currently approved and being used in over 25 countries. SAIB-SG is also being reviewed by several regulatory agencies. The Food and Drug Administration in the United States has been actively reviewing SAIB-SG for several years. The European Union Scientific Committee for Foods has reviewed SAIB-SG, and assigned it an E number, E-444. It is one of the ingredients awaiting legislation before full approval can be obtained in Europe. The Joint Expert Committee on Food Additives has already reviewed SAIB-SG and established an acceptable daily intake of 10 mg SAIB-SG/kg body weight. The advantages of SAIB-SG include; it is a high purity distilled product, it is odorless and flavorless at levels used in beverages, and where it is allowed it is regulated at the highest level of the density adjusting agents. The most commonly voiced disadvantage of SAIB-SG is the physical form. SAIB-SG is a viscous liquid which requires heating to improve its flow properties. The viscosity of SAIB-SG at 30°C is approximately 100,000 centiPoise (cP). By heating this material to 70°C the viscosity drops to 1,000 cP, and it becomes a free flowing liquid.

Heating SAIB-SG is not always a practical approach when dealing with beverage production. Another method of lowering the viscosity of SAIB-SG is to dilute the material with another food grade product. Ethanol is an excellent solvent and viscosity reducer for SAIB-SG. A blend of 10% ethanol and 90% SAIB-SG is a free flowing pumpable liquid at ambient temperatures.

Let's turn our attention from the ingredients used to how they are used. For typical orange cloudy beverages the oil phase needs to be adjusted to a specific gravity between 1.01 and 1.03 to achieve a stable beverage. One method used to determine the ratio of density adjusting agent and essential oil needed to provide a given specific gravity, is the Pearson Square Procedure. The Pearson Square procedure requires that you know 3 pieces of information; the specific gravity of your essential oil, and the desired oil phase specific gravity. These values are arranged as shown below:

#### PEARSON SQUARE PROCEDURE # 1

<u>Ingredients</u>	<u>Specific Gravity of Ingredients</u>	<u>Desired Oil Phase Specific Gravity</u>	<u>Volume Ratio of Ingredients</u>
Density Adjusting Agent	SG1		?
		SG	
Essential Oil	SG2		?

The specific gravities of the ingredients are cross subtracted with the desired oil phase specific gravity, smaller number from larger number. The result of these calculations are the respective volumes of density adjusting agent and essential oil required to theoretically give the desired specific gravity. For example, if a specific gravity of 1.015 were desired, and SAIB-SG and essential orange oil are used, this is how the calculation would be performed (see below, pearson square procedure number 2):

### PEARSON SQUARE PROCEDURE

<u>Ingredients</u>	<u>Specific Gravity of Ingredients</u>	<u>Desired Oil Phase Specific Gravity</u>	<u>Volume Ratio of Ingredients</u>
SAIB-SG	1.14		0.175
		1.015	
Essential Oil	0.84		0.125

or 175 parts by volumes SAIB-SG plus 125 part by volume essential orange oil will produce an oil phase specific gravity of 1.015.

After the SAIB-SG/flavoring oil have been combined they are incorporated into a beverage emulsion. A potential orange emulsion formula is listed below.

### Orange Beverage Emulsion Formula:

#### Ingredients:

Food Grade SAIB-SG	100 g
Orange Flavoring Oil	050 g
Emulsifier	
Acacia Gum	150 g
or Modified Starch	120 g
Preservative, Potassium Sorbate	001 g
Acidulant, Citric Acid	001 g

This emulsion is then homogenized to obtain the correct particle size distribution, > 90% less than 1 micron in diameter. These emulsions can then be shipped to remote locations where beverage syrups and finished beverages are prepared.

In conclusion, Food Grade SAIB-SG is an excellent density adjusting agent for cloudy citrus beverages. The stability and flavor profiles are very good. And when the FDA and EC regulatory approvals are completed, Food Grade SAIB-SG will become the only density adjusting agent has regulatory acceptance in most portions of the world.



# GRAIN HANDLING, STORAGE AND DISTRIBUTION IN THE PHILIPPINES

*Silvestre C. Andales, Ph.D., Edgardo S. Manebog and  
Manolito C. Bulaong.*

*National Post Harvest Institute For Research and Extension, Department of  
Agriculture, Philippines*

## ABSTRACT

Rice and corn production in the Philippines has been falling short of demand due to several calamities visiting the country resulting in successive importation of these commodities in the past years. At present, post-production operation has yet mechanized. Mechanization cost is too high for the average farmer. Harvesting of crops is still done manually although mechanical reapers and strippers are available in the market. Most drying operation is still by sundrying, although some government agencies and a few trader-millers use mechanical drying facilities. Milling of rice is now fully mechanized. The total capacity of rice mills exceeds the total rice requirements. Present storage capacity is also sufficient for up to the. Bulk handling and storage of grains still has to be studied.

To meet the growing needs of the country, the government has launched the Grains Production Enhancement Program (GPEP) aimed at increasing production to 12 and 17 million MT for rice and corn respectively by year 1997-98. Concerted efforts to provide high-yielding (HYV) seedlings, farm inputs, post-harvest facilities, financing as well as training needed by recipient cooperatives to realize the vision of the program, are being done by the various government agencies.

## 1. INTRODUCTION

The Philippines is basically an agricultural country but in 1973 the country experienced rice shortages. However in the early 80's, the country recovered and even exported rice surplus. This increased productivity was attributed to the introduction of high yielding rice varieties, improvement of farm irrigation, financial facilities, and technical as well as marketing services extended to the farmers. This boom years was short-lived.

By mid-80's, there, was again rice shortage. Programs of different government agencies were focused towards empowering the farmers. The National Food Authority (NFA) originally mandated for stabilization, regulation and market development of the grain industry was re-oriented to be a service corporation catering to the needs of the farmers. The Department of Agriculture (DA) helps not only in crop production but also in the establishment of farmer cooperative which were envisioned to be in trading and processing. The Grains Production

Enhancement Program (GPEP) currently being implemented by DA is a total package designed to increase rice and corn production by the year 1997-98.

To implement this program the current post-production operations needs to be improved. This calls for upgrading the mechanization level of farming, educating the farmers, and retooling or upgrading the knowledge of farm technicians regarding post-harvest technologies. Necessary post harvest facilities has to be established and those existing made available to farmers.

## 2. HARVESTING

Survey of existing practices (19) reveal that only about 58% of the farmers harvest their crop at maturity, 7% before maturity and 35% after maturity. Harvesting at maturity and after maturity leads to significant amount of losses. Due to difficulties in handling wet grains at farm level, decision to harvest is usually influenced by weather conditions. On-farm losses (11) range from 2-7%, including shattering, weather damage, pest infestation as well as spillages during handling.

Harvesting of paddy at present is done manually, although mechanical reapers and strippers are available in the market. The traditional tools being used by farmers are yatab, lingkao, and sickle (Fig.1). After cutting the paddy, the rice straw is laid on the stubble and allowed to dry to about 16%. Afterwards, it is gathered and piled for threshing. The adoption of small mechanical threshers (0.5-1.5 tph capacity) replaced the large McCormick type threshers used earlier.

Mechanical reapers (Fig. 2) and strippers are commercially available but require high investment cost. Strippers, unlike reapers, strip the grains right at the ground level without the need for separate cutting and threshing operations. They are gaining popularity because of its simpler mechanism. In-field losses are observed to be less than 1%. Comparative performance of the harvesting methods is presented in Table 1.

Practices for corn harvesting vary from region to region, but, this operation is generally done manually. In southern Philippines, corn is harvested at about 24-30% MC and shelled right away without pre-drying, while in the northern part of the country, farmers practice field drying before shelling, hence shelling is easier and the shelled maize is cleaner than those without pre-drying. Mechanical harvesters are not used because farm sizes are small.

## 3. THRESHING AND SHELLING

Threshing of paddy is done at the field both by manual and mechanical means. Manual threshing is accomplished using hampasan, feet threshing, or by trampling using animals (Fig. 3). The threshed paddy is cleaned by winnowing.

Several types of mechanical threshers of different capacities are available and adopted by farmers. This includee the pedal type and engine powered threshers (Fig. 4), the most common of which are the IRRRI-designed axial flow thresher (Fig. 5). The material is fed between the revolving cylinder and the stationary concave goes around the cylinder axially and is discharged at the end of the ma-

chine. Another type though less effective type is the flow thru thresher (Fig. 6) where the material is fed between the revolving drum and stationary concave, and goes straight out of the machine. Other machines have double drums to increase the separation efficiency but requires higher power than single drum type. Generally, threshing efficiency of mechanical threshers are affected by the length and moisture of the straw, feeding rate and drum speed.

The use of mechanical threshers gives the farmers the opportunity to gather their crop quickly and conveniently with less cost. Different locally manufactured threshers and their performances are show in Table 2.

The traditional method of shelling corn using Gadgaran (Fig. 7) is disappearing due to the proliferation of mechanical shellers in corn producing areas. Mechanical corn shellers, when classified according to the mode of shelling, maybe in crushing or non-crushing type. Crushing type shellers use a rotating drum, with helically-arranged peg-tooth and a stationary concave drum. This type of sheller is produce crushed cob with the output. It has a relatively higher power requirement than non-crushing type but has higher capacity and flexibility with regard to the quality of the input cobs. Shellers may also be of stationary or traveling type. Shellers that can shell ears with husk are the newest type available in the market. A lower capacity is expected for this type since the machine combines the dehusking and shelling operations simultaneously in a single drum.

Like the threshers, corn shellers are locally manufactured except for the prime mover. NAPHIRE (1986) have developed a utility mode of corn sheller (NICS) and have since been commercially adopted (Figure 8). Types and performance of existing shellers are shown in Table 3.

#### 4. DRYING

Most of the grain produced in the Philippines are sundried although mechanical dryers are also available. Mechanical grain drying is not attractive for individual farmers due to the following reasons: high fuel cost, high investment cost, mismatched capacity with the user's requirement and, difficulty in mixing the grains to prevent uneven drying (17). Farmers and traders, who sundry on concrete pavements, including highways, pay only the daily wage and food for laborers, and the transport cost. A study conducted by NAPHIRE (18), showed that highway drying losses could be as high as 2.6%. Considering the average losses of 1.7% for wet and dry season harvest for Central Luzon alone, the losses is estimated to be about 19,765 metric tons if 50% of the production in 1989 is dried on the highways. Aside from quantitative losses, there is a significant increase in quality losses (Table 4) such as cracked grains resulting to about 2.3% reduction in milling recovery of the paddy.

Highway drying also poses hazards to motorists and pedestrians. This prompted NAPHIRE in 1992 to campaign to prohibit the use of roads for sundrying. With the help of the local government units, highway drying was banned in Nueva Ecija and is starting to be enforced in neighboring provinces. Local government officials recognize the importance of not only decongesting the highways but also

the benefits from high quality mechanically dried grains. The campaign was accepted by the concerned farmers and traders and it was envisioned that this move will be duplicated by other regions nationwide. Total success of this program depends on the alternatives that can be offered. Traders may be able to afford the high cost of investment for a mechanical dryers but the farmers, can only availed of the equipment by way of a cooperative. The types of mechanical dryers available in the market as listed in the Table 5.

The NFA and some big millers use mechanical dryers. A significant number of mechanical dryers (flat bed and flash dryers) are now being distributed to the farmers' cooperative as a part of the government's Grain Production Enhancement Program (GPEP) post harvest component. The program aides to distribute about 8,259 units of mechanical (flash) dryers up to 1988 (7). These flash dryers, developed at NAPHIRE, operates at high temperature to dry grain down to about 18% MC at a rate of 600 kg and 6% moisture extraction per hour. At this moisture level grain can be temporarily stored for about three weeks while waiting for the availability of sundrying. The flash dryer can also operate as a recirculating batch dryer for drying down to 14% MC (Fig. 9).

GPEP will also grant a total of 6,345 units of multi-purpose drying pavements measuring 420 sq meters (80-100 capacity) for the cooperative.

## 5. MILLING

Rice milling in the country is almost fully mechanized. The traditional hand pounding (mortar and pestle) in remote villages are fast disappearing due to the availability of rice mills. Out of the total mill polpulation of 13,579 units, it was estimated that Engelberg mills (steel huller) represent 40%, Cono mills 22% and rubber rolls 38%. This is equivalent to 19%, 36% and 45% share of the total milling capacity in cavans per hour, respectively (Figs 10 and 11).

The government regulates use of ricemills through NFA. Owners are required to register their rice mills. The minimum recovery required is 62%. Issuance of licenses also considers the number of mills. Applicants in the area to ensure economic viability. Based on presently registered units, it was found out that rice mills are under utilized (10). Based from the available volume of paddy (9,040,807 MT in 1991) for milling and the total milling capacity of 12,017,600 MT, these rice mills are underutilized by about 2 hours in an 8-hour or 1,600 hrs of operation per year. Comparative performance of these milling systems are presented in Table 6.

Continued use of the steel huller results in enormous losses (approx. 7%). From the study of NAPHIRE (6), it was estimated that in 1991, about 10.2% of total paddy produce was processed by village type rice mills which are mostly the steel hullers. Had this volume been procesed by high performance mills at 62% milling recovery, the country could have saved about 55,311 MT if milled rice or 11.06 M US dollars.

### Village Type Rice Mills

In villages, the most common system of milling is “kiskisan” or steel huller (Fig. 12). Originally designed as coffee mill, these steel hullers were used and adopted for rice milling. Engelberg rice mill consist of a fluted rotating drum enclosed by steel screen. Dehulling and whitening process occurs inside this single drum. This type of single pass mills is noted for its poor grains recovery and high bran yield. There are several attempts to improve the performance of this mill, the most promising is the integration of a rubber roll as a hulling head and utilizing the steel huller as a whitener. The modified system or Improved Village Rice Mill (Fig. 13) is now for dissemination. However, the cost of the modified system approaches that of the two-pass rubber roll mills available in the market.

The use of small or single pass rubber roll mills (Fig. 14) is now becoming popular in the villages and has started to replace the steel huller. This type uses a rubber roll dehusker and a horizontal abrasive whitening unit beneath. Some units are set on mobile platforms so it can go around individual form of creston milling.

### Commercial Type Rice Mills

Commercial milling and trading are usually found in the towns. They supply rice to wholesalers and retailers.

The traditional machinery used by commercial millers like the “Cono” mill system (Fig. 15) consists of an underrunner stone disk huller in combination with a series of cone type abrasive whitener. The paddy separating machine is a huge tray type that occupies a significant space. This system perform quite better than the steel huller. Although system components are quite bigger yet they are a bit cheaper than the modern types of rubber rolls multi-pass mills. To date, these conventional “cono” system are now being modified with rubber rolls as huller or integrating friction whitener in the system and others.

The latest milling system introduced in the industry is the rubber roll multi-pass system (Japanese milling technology) (Fig. 16). This type of modern milling system which were imported are usually found in government grain centers (NFA). It includes the state-of-the-art in rice milling such as automatic (self-adjusting) rubber roll hullers, auto weighers, tray type paddy separators, a series of abrasive and friction whiteners, gyro sifter, rotary length graders and color sorter. The system is operated by remote control. Some privately owned rice milling complexes use this modern type of system such as the Meralco Corfarm. They have established grain centers in rice producing provinces. These grain centers serve as procurement stations where dehulling is also done. The brown rice is then transported for further processing and packaging to a center located just outside Metropolitan Manila.

## Corn Milling

There are two types of corn milling (4) in the Philippines, one is dry milling and the other is wet milling. For dry milling, the products are corn grits and flour, and for wet milling the product are corn starch and flour. Government policy on corn milling limits the operation of wet mills to about 1/3 of their plant capacity which means that processing of corn for industrial use is of low priority.

Several types of corn mill are used in the industry. These are classified into grinder, roller and hammer mill types. Grinder types (Fig. 17) are very similar to the Engelberg mill. It consists of a steel screw that pushes the material to a pair of grinding plate. They are simple in design and low in cost. This type can only mill 30-60 cavans 12 hour operation, hence they are usually found in the villages.

The Hammer mill (Fig. 18) is an all purpose type which can be used for most cereals such as sorghum and soybean to produce livestock feeds. This type of mill consists of several rapidly revolving hammers which pulverize and force the ground material to pass through grinding levels or screen.

Roller type mills (Fig. 19) offers a little sophistication and is commonly used in commercial operation owing to its high capacity (70-120 bags/12 hr. operation). This type of mill is provided with a degerminating machine prior to final grinding of the corn using a set of steel rollers or sets in a series.

## 6. GRADING

Grading and pricing is very subjective. The quality of grain is determined through visual inspection or hampas method (for rice). The latter is done by putting a small sample of rough rice into a piece of cloth and striking it into a hard surface. This method dehulls the rough rice, allowing visual inspection of the brown rice. Moisture content is by feel method or grit wherein grain is either pressed with the thumb nail (for corn) or by biting the grain. Moisture meters are rarely used and there are no existing grading equipment to separate good grains from the damaged ones (13,21). Discounts are subtracted to the gross weight according to the degree of quality deterioration (moldy, discolored, damaged), product form (ear corn or shelled corn), and moisture content (wet, semi-dry, and dry). Mechanical damage or cracked grains do not affect the grading of corn as long as it is dry. The local trader's reason for this is that these grains will eventually be converted into feeds or corn grits.

NFA pricing and grading is primarily based on moisture content and purity of the grain. Other factors include damaged, moldy, shriveled or immature grains, and grains with other color. In grading, the NFA uses the equivalent net weight factor (ENWF) table designed in procuring commercial grains. Under this system, deductions in weight are imposed depending on the MC and purity of the grain (13). Standard for paddy grading is presented in Table 6.

Except for NFA a few private millers who supply big supermarket, and restaurant. These millers also equipped their facility with rice graders to assure the highest possible percent head rice for premium price. Generally, consumers are not strict with respect to the percent head rice that goes to the table but they are more conscious is the whiteness and aroma of the rice. Table 8 presents the NFA grade requirements for milled rice.

NFA standard classification for corn grits includes only Grit No. 10, 12 & 14, (Table 9). However, other existing standards include grit Nos. 16, 18, 20 and 24.

## 7. STORAGE OF CEREAL GRAINS

Cereal grains are produced seasonally. Harvesting is normally done in two to three months thus temporary storage is necessary.

Storing food grains is important to effect a uniform supply of food throughout the year, either for home consumption, domestic or export market; and to provide a reserve for contingencies such as droughts, floods, and war. Some entrepreneurs store food grains to speculate on good price either in domestic or in the export market.

Quantitative losses in storage of rice has been estimated to range from 2-6% (17). In 1977, the Food and Agriculture Organization (FAO) estimated the worldwide loss in corn storage to be about 13%. These losses are due to factors such as use of traditional practices and inadequate facilities in grain storage resulting in spillage, infestations by birds, rodents, insects, molds and dry matter loss due to respiration.

While new techniques have been developed storage facilities are not sufficient to increase productivity. This was the case during the previous government programs of MASAGANA and MAISAGANA wherein the resulting increased production were not accompanied by improved postharvest facilities such as dryers and storage infrastructure.

### Existing Practices

Figure 20 summarized the different types of farm level storage. There are several types of commercial-level storage presently used in the grain industry, namely:

1. Warehouse - This is usually a concrete building with galvanized iron roofing provided with roof ventilator. Wooden pallets hold the sacks of rice.
2. Flat Store - It is similar to a warehouse but the grains are stored in bulk rather than in sacks.
3. Rectangular steel/wooden bin - It is normally used for bulk storage of grains inside the building.
4. Steel/concrete silos - These are tall cylindrical structures for bulk storage of grains.

Figure 21 and 22 illustrate the relative positions with respect to the post harvest system of rice and corn respectively.

### Principles of Cereal Grain Storage

The primary concern of storage is the safety of the product. Temporary storage of cereal grains before the drying operation is critical because deterioration of the product at high moisture content is normally very high. For medium and long term storage both the quality and quantity of the stored product have to be maintained. To achieve this purpose, the five aspects of storage have to be considered, namely: the product to be stored; the storage structure; the storage pests; the environmental factors; and the personnel requirement.

#### The Stored Product.

Grains after harvest continue to respire, generating heat carbon dioxide and water in the process. The build up of carbon dioxide displaces the surrounding oxygen causing a slowing down of the respiration process. But, the moisture generated increases the moisture content of the grain resulting in microbial and fungal growth. The amount of carbon dioxide is usually measured to indicate the extent of the respiration (deterioration) process. The generated carbon dioxide and water are given off to the ambient air as a dry matter loss in the stock.

Table 10 and 11 show the carbon dioxide, water, and heat generated and the dry matter lost from 1000 tons of paddy and corn respectively, at different moisture contents in a one day time period. As shown a warehouse with a capacity of 1000 tons of paddy will generate 10.32 kg of carbon dioxide, 4.22 kg of water, and 110,080 kg of heat in one day. On the other hand, as shown in Table 11, corn will generate 20.24 kg of CO<sub>2</sub>, 11.55 kg of H<sub>2</sub>O, and 300,800 kJ of heat.

Both the generated water and heat from respiration can be removed through ventilation or aeration. The above indicated figures are the parameters in the design of aeration systems.

In bulk storage of grains, the amount of water and heat is proportional to the storage period.

Processed grains (milled rice and corn grits) do not respire so much. However, because the protective covering such as the rice hull and bran in the case of rice and the pericarp in the case of corn are already removed, they become more vulnerable to the attack of insects and molds. The milled products have short shelf life.

#### The Storage Structure/Container.

The storage structure/container protects the product from the unfavorable environment and pests. The warehouse should be properly kept, secured, and maintained. The storage structure should be well designed, strong and weather-

tight to protect the stored product from inclement weather. It should have aeration and fumigation facilities.

### The Storage Pests

The storage pests could be insects, rodents, birds, and molds. The prevention and eradication measures against these storage pests are necessary and are well established in storage operations. In practice, eradication rather than prevention is common measures to prevent pest infestation may include:

#### 1. Sanitation

The most important and effective preventive measure is sanitation. About 50% of the problems in pest control is solved by observing and enforcing cleanliness. Sanitation could be achieved by requiring systematic arrangement of stocks; providing ample space for movement of personnel; requiring strict implementation of rules and regulations; providing ample supply of cleaning tools and materials; and conducting regular inspection of the stocks and facilities.

#### 2. Legislation

National quarantine law dictates that some products are completely prohibited entry while others are allowed only after rigorous inspection. Pest prevention can be addressed by strictly enforcing the country's quarantine laws applicable to grain importation.

#### 3. Disinfestation

The storage structures and containers should be disinfected before a new stock of product is received for storage. This is done by spraying, dusting, fogging, and fumigating. When the stocks are already infected with storage pests, they can be eliminated or their growth arrested by chemical, biological, and/or environmental control.

### Mold Infestation in Corn

A very serious pest in storage of corn is the molds or fungi. Two most important storage fungi in corn and other agricultural products are the *Penicillium* and *Aspergillus*. Storage fungi can cause grain damage when condition for their development are favored. The deleterious effects on grains are as follows:

1. Decrease in germinability.
2. Altered nutritional value.

3. Heating of the product while in storage.
4. Discoloration of the product.
5. Caking of grains in storage
6. Low milling yields.
7. Health hazards.

The most serious effect of molds is the production of mycotoxins. Mycotoxins, when cause serious health risks. The most potent of the mycotoxins is the aflatoxin produced by the fungus *Apergillus flavus* and *Aspergillus parasiticus*. Findings of NAPHIRE researchers revealed that about 70% of the corn in the different trade channels in the Phillippines are contaminated with aflatoxins above the tolerable level of 20 ppb. Drying, aeration, proper effective means of preventing fungal damage.

#### Environmental Factors Control.

The hot humid climate in the tropics is not suitable for grain storage. The average relative himidity is about 80%. While, the requied relative humidity for storage is about 70%.

The temperature and relative humidity varies within the year. About six months of the year the air is too humid. In addition, the stored product acts heated no. by absorption of heat from the atmosphere and from its own heat of respiration rate. The molds and insect pests rapidly multiply increasing the heat further. When the heat is not removed and the temperature becomes excessive, heat-damaged kernels are produced.

To protect the stored product from these factors, the storage structure must be provided with good ventilation and aecration system.

#### Personnel Requirement

Personnel are needed to take care of the storage operation, to maintain and repair the storage structure, to prevent and control pest infestation, to maintain proper temperature and humidity conditions; and most of all to monitor the quality of the stored products.

The following personnel are needed in a grain storage set up:

1. Engineers, to take charge of the storage and the environmental problems;
2. Entomologists to take charge of the biological aspects;
3. Technicians, to carry out the operation, maintenance, and repair;
4. Managers, to orchestrate the various management and liason functions.

#### Stock inventory

An inventory system should monitor the stock at all times changes in quality of the product inside the warehouse are due to receipts and deliveries (issues) of

stocks and losses caused by respiration, microorganisms, insects, rodents, birds, spoilage and change in moisture content.

In normal warehousing practice, different types of products are stored in one storage building. Grains with varying moisture contents, and quality grades are sometimes stored together in one building. These mixtures require the adaptation of a systematic segregation system. Stocks should be properly labelled with all relevant information. This will facilitate inventory monitoring and allow the application of the first in-first-out (FIFO) rule. The bulk storage, it is not advisable to mix commodities just to maximize storage capacity.

### Storage Capacity

Adequate storage structure is capital intensive. It should be of optimum size to hold the grains without incurring deterioration. In the case of rice, paddy or rough rice keeps longer than milled rice but, milled rice requires less storage space so a balance should be made between the capacity for paddy storage and the capacity for milled rice storage. This will determine the size of the rice mill to maintain.

## 8. TRANSPORT, HANDLING, AND MARKETING OF GRAINS

### Supply and Demand

In 1992, total production of palay is 9.1 million metric tons (16). Luzon contributed to 62% of the total rice production, while Visayas and Mindanao both contributing about 19% each.

Corn supply is concentrated in the northern and southern regions of the country. In 1990, corn production was 4.9 million metric tons (14), Mindanao accounting for 67% of the total production volume, while Luzon and Visayas contributed 22% and 11% of the production volume respectively. Supply is greatest in the second half of the year, accounting for 78% of the total production as an effect of peak rainfall during this period.

Ninety-one percent of rice production is biggest in Luzon, it is also the biggest consumer, taking about 58% of the total consumption, followed by Visayas and Mindanao, which consumed 22% and 21% respectively.

The rice being sold in Metro Manila markets, the largest rice-deficit area, come from Tarlac and Nueva Ecija and Isabela.

The major demand areas for corn are located outside the main production areas. Since the highest concentration of feedmill, hogs and poultry is in Metro Manila (NCR), Central Luzon (Region 3) and Southern Tagalog (Region 4), these regions together received 96%. Manila is the destination of 92% of yellow corn shipments from General Santos City and 56% of the shipments from northern Mindanao. On the other hand, Cebu received only 8% of yellow corn shipments from General Santos but 37% of shipments from Cagayan de Oro (11).

The biggest demand for white corn comes from Cebu which receives 54%

of the white corn grains coming from Southern Mindanao and 66% of the same coming from Northern Mindanao.

## **Marketing Channels**

### **Rice**

The marketing of rice in the Philippines is done by both the government, represented by NFA, and the private sector. The NFA procures and distributes rice primarily for price stabilization purposes. During the last 10 years (1982-1992), NFA procurement ranged from 2.2% (1989) to 7.8% (1982), while distribution ranged from 1.2% in 1986 to 10.9% in 1983 (16). The private sector consist of rice traders, commission agents, rice millers, wholesalers, and retailers. Farmers sell rough rice either to NFA or to rice traders, which the latter then sell to rice millers. The rice millers dry, store, and mill rough rice, then transport the milled rice to wholesalers and retailers. Retailers sell rice to consumers and institutional clients. These marketing agents sometimes assumes a combination of roles. Sometimes, the rice millers hire commission agents to buy directly from farmers. Wholesalers may also engage in transporting and selling milled rice to other wholesalers in deficit areas. They may also buy rough rice directly from farmers or rice traders, custom-mill, and sell it to retailers (See Figure 23). In Metro Manila, rice is traded by Chinese traders in Dagupan Street and Binondo area. These traders obtained their supply from millers in Tarlac and Nueva Ecija. They have established good business relationships with these millers and new entrants in the market would find difficulty in competing with the traders.

Based on 1992 national estimates (16), farmers sold 41% of their produce, 27.6% was retained for 10.5% given to landlord, 4.4% used for seeds, and 16.2% for other uses. In Cagayan Valley, and Central Luzon, farmers sold 51.7% and 53.5% of their produce respectively. In a survey made by Wedgewood and Duff (21) in Nueva 63.6% of farmers sell their produce to buying stations, 30.3% to rice millers, and 6.1% to NFA. Farmers (82% of those surveyed) immediately dispose their produce within one week after harvest.

### **Corn**

The farmer sells his corn either wet or dry, shelled or in cobs to traders and some to NFA. The operations of a trader depends on the form of the commodity he purchases. A trader's operation may begin with the hauling of the stocks from the farmer to his buying station. The stocks are then classified, weighed and stored. Shelling and drying are done if needed. Storage is temporary until the stocks are transported and sold to the wholesaler. The wholesaler does the same operation as the local trader on a large scale except that shelling is not included in his operation. He then delivers the stocks to processors in Manila or Cebu through hired shipping

vessels.

According to a survey made by NAPHIRE (13) in South Cotabato (one of the main corn producing area) farmers sell about 88% of their produce. The local traders buy 51% of these and the remainder is sold to farmers organization (10%) and to wholesalers (17%). The NFA buys about 4% from the farmer's organization. The wholesalers distribute about 50% of these produce to Manila processors and about 34% to Cebu Processors (See Figure 24,25,26).

### Transport

Grains in bags are hauled manually at all distribution levels. Laborers are paid at the rate of P0.25 to P0.55/move to haul a bag of shelled corn. The transport of corn from the farm to the trader's buying station vary from animal drawn carts, tricycles, trailers, to trucks depending on the distance to be traveled and volume of stocks to be transferred. Large trucks of capacities ranging from 100 to 400 bags are used by traders and wholesalers alike. In South Cotabato, P0.50 kg is charged for trucking corn when stocks are picked up from any place of the trader's municipality to his buying station, while P0.10-0.15/kg is charged for trucking when stocks are transported to General Santos City (13).

Passenger and cargo ships are used in transporting corn to Manila and to Visayan provinces. Passenger ships carry around 8,000 to 10,000 bags of grains while cargo ships can accommodate around 1,000 to 1,200 tons per shipload. Even if there is a regular schedule for ship's departure, this is not usually followed. A cargo ship will not leave General Santos City unless it has attained its full capacity. Travel time to Manila takes around five days while Cebu takes around three days. The stocks going to Manila are placed in 20 or 10-foot containerized vans while those bound for Cebu are piled in pallets (30 bags/pallet). A 20-foot van carries around 200 bags of grains, double the capacity of a 10-foot van. Freight expense of P10.92/bag for grains and P10.15/bag for grits are charged for shipping. Other costs are arrastre, stevedoring and insurance. Yellow corn grains are mostly shipped to Manila while white grains are sent to Cebu (13).

### Handling

Grains are handled in bags or bulk depending on transport, storage and handling facilities available. Overland transport is accomplished by trucking grains in bags, with the loading and unloading accomplished manually by hired laborers. Containerized vessels, roll on- roll off (RORO) and break-bulk tramp vessels only ship grains in bags. Barges ship grains either in bags or bulk. Loading and unloading to and from these vessels are performed by stevedoring service firms which provide the labor and equipment. The type of equipment used varies depending on the mode of loading and unloading. RORO vessels load and unload horizontally over ramps using either manual labor or forklifts. Shore or ship based cranes are used for vertical loading and unloading bags of grains placed in pallets or tied by the straps. The sacks of grains are reloaded to trucks on the shore for final

delivery.

Corn grains can also be handled in bulk tramping vessels. Bags of corn grains in pallets or straps may be loaded vertically by cranes onto the vessel where they are opened and emptied. In another way, dump trucks and pay loaders are also used to load corn grains directly through a bulk-loading spout that empties into the vessel. Unloading from bulk-handling vessels depend whether the corn grains will be bagged or not. When bagging of corn grains is required, it is usually done manually inside the vessel. The bags of corn are then unloaded vertically in pallets or straps by crane. When no bagging is required, the corn grains are transferred by a clam-shell equipment directly from the vessel to dumps trucks stationed at the shore. A private port in Cebu operated by an integrator can unload corn grains from barges by suction. On the other hand, the dumps trucks that carry corn in bulk from the ports deliver the grains to feed mills that maintain storage silos. The grains are directly emptied into hoppers where they are conveyed by bucket-type conveyors into the silos (11).

### Consumer Preferences

Prevailing rice prices in supermarkets are relatively higher than prices in the public markets (1). The difference in price is due to the better quality of rice sold in supermarkets. Rice are classified fancy, special and ordinary refective quality characteristics. There is a big difference in the price of the fancy grade rice and ordinary variety sold in the public market.

### Effect of government price intervention

To hold prices within the support ceiling price, NFA procures, processes, and stores rough rice, and imports and distributes milled rice. Typically, procurement is done during surplus periods, while rice injections are needed in time of deficit. NFA's performance is a mixture of success and failure, the latter generally caused by unfavorable weather, delayed arrival of imports, and lack of government funds. Generally, the government is more successful in keeping the price ceiling on rice than in defending farm-level support prices. Annual average farm prices remained below average support prices in 12 out of 13 years of procurement (1974 - 86). Lack of funds NFA procurement tro a mere 4.8% of production in 1986. This problem was aggravated when the government lifted NFA's monopoly on wheat and feedgrain import in 1986, from which it generates funds for its operation, forcing the agency to rely more on government subsidies to finance its operation. The procurement is also ouat of timing, i.e. to achieve maximum impact on farm prices, procurement should occur during peak harvest when supply is at its peak. However, based on 1980-86 procurement data, NFA procurement occured after peak harvests. This was primarily due to budgetary problems. Farmers were also reluctant to sell their produce to NFA beacuse of many requirements, delayed payment, and late procurement. NFA's campaign to keep retail prices from rising above the ceiling prices also created a price lid

which also meant significantly greater purchases were required to keep rough rice prices above the support price (20).

#### Implication of GATT-Uruguay Round Agreement on importation of palay and corn.

The Philippines is a member country of the General Agreement of Tariffs and Trades (GATT) since 1980. Recent multilateral negotiations held in Uruguay in 1986 made GATT more comprehensive and integrated new areas including agriculture. Under the GATT on agriculture, all quantitative restrictions will be lifted by member-countries and converted to tariffs. The tariff rates would be equivalent to the level of protection enjoyed prior to the removal of restrictions. Due to food security concerns, developing member-countries like the Philippines have been allowed the flexibility of retaining quantitative restriction for staples. The Philippines chose to retain import restrictions on rice for the next ten years. In exchange for this privilege, the country will be required to allow importation of rice equivalent to 1% of domestic consumption in 1995. This level of importation will increase to 4% in year 2005.

For agricultural products whose quantitative restrictions will be lifted, the Philippines will bind or set maximum limits on tariffs at a minimum of twice the existing tariff rates. For critical agricultural commodities like corn, the bound tariff rate will be increased to 100% from the current rate of 20%. These bound tariff rates will be reduced to levels equivalent to at least ten percentage points higher than the existing rates within ten years starting 1995 (DA, 1993).

#### Problems and Constraints

##### a. Seasonal Corn Supply

Corn production continues to be short of demand forcing importation. Production is also seasonal, a shortage in the first half of the year and a glut in the second half. This kind of situation adversely affects the output of the feed milling and animal industry especially during the period where the demand for hog and poultry is high. This also creates erratic supply pattern and unstable prices resulting to uncertainties in the animal industry. The seasonality of corn harvest also creates shortages of laborers of post harvest facilities and of transport. Such shortages causes unnecessary delays in harvesting, shelling, drying, transporting and marketing of corn with adverse consequences in the price, grain quality and income of farmers. The bumper harvest in the second half of the year also dampens the price of corn, decreasing the farmer's profits (11).

##### b. Poor transport infrastructure

Poor transport infrastructure in rural areas is a cause for delays delivery of agricultural commodities in the Philippines. About 92% of the country's road

network are earth and gravel roads. The conditions of these roads worsens during the rainy season.

c. Grain mixing

Grain mixing at the local trader and wholesaler level is a common and acceptable practice (13,21). Grains of different moisture content are mixed in order to attain the required moisture content level of wholesalers. Likewise, good quality grains are usually mixed with moldy grains, to attain the least required level of the wholesalers. The wholesalers also practice mixing to reach the minimum quality level (97%) that would command the same premium price of good quality grains. For corn, this practice allow mixing of aflatoxin-contaminated grains with good ones,, resulting to high incidence of aflatoxin contamination in the market, even above the FAO maximum limits of 20 ppb (13).

d. Poor market integration

Farmgate, wholesale, and retail market should be integrated so that farmers get feed back on quality preferences of consumers. Studies on market integration from 1974-1982 (20) reveal weak links between farm and wholesale markets. Market integration improved in 1983-86 which was attributed to less government intervention in the marketing system. The farmers are more willing to quality-improving technologies if they have price incentives for doing so level. The price stabilization policy of the government which favors low consumer prices over the propping up of prices, created a "price lid", resulting in lower farm prices and incomplete transmission of consumer preferences.

e. Lack of price incentive

Another problem is the lack of suitable price incentives. The current prices indicate a lack of incentive for farmers to deliver clean, properly dried paddy to the miller of trader. The price paid to farmers for their rice is dependent only to moisture level and other quality attributes (21). Buying and selling on the basis of subjective personal judgment (i.e physical attributes such as color, aroma, variety, etc.) contributes to the farmers' lack of interest in investing on improvements, particularly those which improve quality but not quantity. Although private farm gate prices do include a price premium for dried rough rice, they do not provide sufficient price incentives to dry if it is harvested at the recommended moisture content level and if shrinkage losses and drying costs are considered (21). The lack of price incentive to dry rough rice at the farm level underscore a defect in the marketing system (21).

## 9. Government thrust and directios on food security

To date, the combined post harvest facilities and equipment for both regis-

tered private and government are presented in Table 12. Many post harvest facilities especially those of small capacity warehouse and machinery are not registered with the NFA.

As shown in Table 12, warehousing capacity of the NFA is about 7 times that of the private sector. Assuming that the government will only absorb 50% of the usual 10-12% of the total palay production, this will still mean that farmers cooperative will have a warehousing capability of 3.5 times more than private traders. For mill requirement small milling capacity can be extended to augment the milling requirements of the cooperatives.

With the combine warehousing capacity of both private and government totaling to 21.4 million tons, the country can still accommodate the projected requirements (11) of 12 million tons up to year 2000 (72 million projected population) with an excess of 9.5 million tons capacity.

The National Food Authority (NFA) mandate to ensure the availability of basic staples (rice and corn) at reasonable prices in all levels of society in all places of the country especially during times of calamities and national emergencies, still remains.

With the change in political order in 1986, the activities of the agency were trimmed and its missions and directions were redefined. Originally, the policy on stabilization was seen or equated to be a direct market intervention. For two decades, the government sets the support price for farmers and low selling prices for the consumers. With these intervention, the amounting subsidy caused the government about 700 million - 2 billion pesos per year.

Today, the major functions (5) of the NFA such as stabilization, market development and industry regulation are being re-oriented to from the farmers. The government envision the farmers cooperatives to become self-reliant and the NFA must transform into a grains service corporation.

To shift from a trading agency to a service corporation (22), the agency should provide facility services such as storage, mill, transport and other post harvest machineries for farmers use. Following are the implementing guidelines:

- NFA is targetting to store, mill and transport about 400,000 MT of palay, rice and corn stocks owned by farmers as a part of facility services.
- Push farm prices to benefit the farmer. NFA shall provide post harvest facilities for the farmer to use. This will control the entry of excess supply in the market.

Industry-Directed Activities, Policies and Procedures. Operational and developmental activities must be geared toward the need of the techniques. The government also supports environmental conservation through such projects as rice hull utilization, waste recycling and grains composting system.

Another major thrust of the government regarding food security (9) is the GPEP which aims to increase rice production to 12 million MT and 7 million MT for corn by the year 1997-98. The country's rice and corn production has been

falling short of demand resulting to successive importation of these commodities in the past years. Just in 1993, the country imported 209,000 MT of Thai rice to augment the domestic supply. In order to increase production, the program targetted to increase the average production of 3.5 MT to MT/hectare for rice, and 3.25 MT to 5MT/hectare for corn by the year 1997/98. These will be possible if 1.2 million hectares were allocated for rice and 700,000 hectares for corn. The program will provide low cost production inputs to farmers in Key Grain Areas. It also should provide irrigation infrastructure support, production technology and training component, financing support as well as post harvest facility including transport infrastructure support.

### BIBLIOGRAPHY

1. Abejuela, A.G. and B.M. Manalansang. In: Naewbanij, J.O. ed. 1993. Increasing Handling, Processing and Marketing Efficiency in the Grain Postharvest System. Proceedings of th 16th ASEAN Seminar Postharvest Technology. Phuket, Thailand. ASEAN Grain Postharvest Programme.
2. Cardino, A.G., R.P.Cabacungan and E.Z Bermundo. 1988. Socio-economic factors affecting the utilization of post-harvest equipment in the maize industry. Grain Protection in Post Harvest Systems. Proceedings of 9th ASEAN Technical Seminar on Post Harvest Technology. Ed. by B.DM. de Mesa. ASEAN Crops Post Harvest Programme. Manila. Phils. pp 315 - 324
3. Chinsuwan, W. 1987. Final Report on: Groundnut shellers/strippers project. Khon Kean University. Department of Agricultural Engineering, Khon Kean, Thailand.
4. Corn Post Production Operations. 1990. The Philippines Recommends. PCARRD Technical Bulletin Series no. 71. PCARRD, Department of Science and Technology. Los Banos, Laguna, pp 1-107.
5. David, R.G. 1992. The New Thrust and Directions of the National Food Authority. National Food Authority. Quezon City, Philippines. pp 25.
6. Geron. L. and Ramos \*\*\*\*\*National Post Harvest Institute For Research and Extension, Munoz, Nueva Ecija, Philippines. \*\*\*
7. GPEP Targets and Budgetary requirements, GPEP Program Document. 1994. Dept. of Agriculture - NAPHIRE, Munoz, Nueva Ecija, Philippines.
8. Grain-Legume Post Production Operations. 1991. The Philippines Recommends. PCARRD Technical Bulletin Series No. 73. PCARRD, Department of Science and Technology, Los Banos, Laguna. pp 1-107.

9. Grains Production Enhancement Program. 1993. Program Document. Department of Agriculture, Philippines. pp 1-10.
10. Gregorio, F.L., A.A. Arcilla, 1993. Policy imperatives for the development of the rice milling industry in the Philippines. Unpublished Report. NAPHIRE Project Review Document. NAPHIRE - DA Munoz, Nueva Ecija Philippines. pp 11.
11. Labadan, M.M. Corn and Feedmilling Industry in the Philippines. In: Naewbanij, J.O. ed. 1992. State of the Art of the Grain Industry in the ASEAN: A Focus on Grain Handling and Processing, Proceedings of the 15th ASEAN Seminar on Grain Postharvest Technology. Singapore. ASEAN Grain Postharvest Programme.
12. Manebog, E.S., R.P. Gregorio and R.E. Manalabe. 1986. Design and development of an improved mobile corn sheller. Terminal Report. The National Post Harvest Institute For Research and Extension. Munoz, Nueva Ecija.
13. NAPPHIRE. 1989. Control of Aflatoxin in CORN (Philippines). Terminal Report (Unpublished). International Development Research Center, Canada.
14. Philippine Agribusiness Factbook and Directory. 1992. Center for Research and Communication.
15. Q & A About GATT and its Implication on Philippine Agriculture. (1993) Published by the Department of Agriculture.
16. Regional Rice Statistics Handbook. 1970- 1992. PhilRice-BAS Collaborative Project.
17. Rice Post Production Operations. 1987. The Philippines Recommends. PCARRD Technical Bulletin Series No. 63. PCARRD, Department of Science and Technology. Los Banos, Laguna. pp-1-107.
18. Tolentino, H. A. Rodriguez and A. Regpala. 1992. Study on the alternatives to highway paddy drying in Central Luzon. Terminal Report. National Post Harvest Institute For Research and Extension, Department of Agriculture. Munoz, Nueva Ecija, Philippines. pp 18-21.
19. Toquero, Z. and B. Duff. 1974. Survey of postproduction practices among rice farmers in Central Luzon. Saturday Seminar Paper. IRRI, Los Banos, Laguna, Philippines.
20. Umali, D.L. B. Duff. The Philippine Rice Marketing System: Implications

for Grain Quality Improvement. In: Naewbanij, J.O. ed. 1990. Advances in Grain Postharvest Technology Generation and Utilization. Proceedings of the 11th ASEAN Seminar on Grain Postharvest Programme. Bangkok, Thailand.

21. Wedgewood, H. and B. Duff. In Unnevehr. L.J.B. Duff. B.O. Juliano. 1992. Consumer Demand for Rice Grain Quality. IRRI. IDRC. 4. Bautista, R.C. 1979. Performance evaluation of the Malaysian Threshing box and the modifications under Philippine conditions. M.S. Thesis. Univ. of the Phils. Los Banos, Laguna, Philippines.
22. Work Program of National Food Authority. 1994. NFA, Quezon City, Philippines. pp 4.

Table 1. Comparative capacities of the different methods for harvesting paddy.

Operation	Capacity
Harvesting	
a. Manual cutting	
1. Yatab	240 manhrs/ha
2. Lingkao	143 manhrs/ha
3. Sickle	60-80 manhrs/ha
b. Mechanical reaper	
1. 1m. model	2.4 ha/day
2. 1.6m. model	3.8 ha/day

Phils. Recommends for Rice, 1987

Table 2. Comparative performance of different locally manufactured threshers.

	TH 1	TH 2	TH 3	TH 4	TH 5	TH 6	TH 7
Gross Weight	722.2	722.5	615.5	758	327	669	-
Engine Hp	16	1	16	12	16	12	16
Conditions of test:		12					
Grain MC, %	15.7		25.7	19.8	14.7	16.6	19.9
Straw Length, cm	51.2	17	50	55	5	57.5	61.5
Straw Grain Ratio	0.87	42.5	1.08	1.05	1.25	1.01	0.98
Performance Criteria							
Noise Level, db(A)	88-92	1.62	89.91	86-	90-92	91-	95-96
Fuel Consump, li/hr	3.82		3.05	89	1.04	92	3.91
Capacity, kg/hr	1240	89-91	1570	2.72	956	2.16	39.66
Purity, %	17.3		98.18	1378	94.25	1182	92.76
Aspiration Loss, %	0.68	2.12	2.88	97.32	0.61	95.47	2.54
Separation Loss, %	0.14	1561	0.50	4.1	0.61	0.70	0.56
Unthreshed Loss, %	-		-	0.31	-	0.47	-
Threshing EFF., %	99.18	90.25	96.6	-	98.6	-	97.4
Broken Grains, %	3.0	-	2	95.58	3	98.8	0.86
Cracked Grain, %	7.5	2.35	2	5	3	4	4.33
		-		19		8	
		0.31					
		-					
		97.3					
		3.5					
		6.0					

TH 1 - Lakas Kuliglig Thresher

TH 2 - Lakas Elepante Thresher

TH 3 - Gintong Ani Thresher

TH 4 - Kanthalux Palay Thresher

TH 5 - Nayon Grain Thresher

TH 6 - Anihan Grain Thresher

TH 7 - Fieldstart 100 TH-8

Source: Ag. Machinery Testing and Evaluation Center (AMTEC), 1983

Table 3. Comparative types, specifications and performances of existing maize shellers.

Specification/ Performance Indicator	Maloloy -on	Summist	Chikuma	AMDP	Mc Cormick	NICS
Type: No. of Drum	Crushing; 2 Drums	Crushing; 1 Drum	Non- Crushing; 2-sided disc	Non- Crushing; 2 drums	Non- Crushing; 1-sided disc	Crushing; 1 drum
Output Capacity, bags/hr.	50	45	3	10	7	40
Prime Mover	16 hp gasoline	12 hp diesel	1 hp Electric motor	5 hp Gasoline	6 hp Diesel	16 hp Gasoline
No. of laborers required, min.	3	3	1	2	2	3
Separation Mechanism	Screen & Blower	Screen & Aspira- tion	Blower	Screen & Blower	Blower	Screen Aspira- tion
Shelling efficiency	95.6	92.9	93.9	97.1	78.7	98.0
Damaged Grains, %	2.5	7.2	4.7	3.8	12.2	1.87
Unshelled Loss, %	0.78	0.59	1.31	0.10	11.5	0.13
Impurities, %	0.50	1.17	0.75	0.40	0.20	0.27

NICS - Naphire Improved Mobile Corn Sheller

AMDP - Agricultural Machinery Development Program

Source: Manebog, E. S. and R. E. Manalabe. 1968

Table 4. Qualitative and quantitative evaluation of mechanically and sundried paddy.

Parameters	Mechanical Drying	Sundrying	Stat. Significance
Impurities, %	2.09	2.84	s
Cracked Grains, %	29.88	35.82	s
Head Rice, %	79.35	71.84	hs
Brokens, %	20.94	28.17	hs
Milling Recovery, %	63.36	61.08	hs
Moisture Content, %	12.79	11.62	hs
Immature/Chalky, %	6.77	7.58	ns
Whiteness, L value	80.57	80.69	ns
Yellow Grains	9.76	9.68	ns

s - significant at 5% level

hs - highly significant (1% &amp; 5%)

Source: Tolentino et. al. 1992

Table 5. Types of mechanical grain dryers available in the Philippines.

Characteristic/ Feature	Flat Bed	Flash Dryer	Continuous Flow (LSU)	Recirculating Batch
Type of bed/bin	-simple flat bed -reversible airflow	-non-mixing columnar with reversible airflow	-mixing type column with inverted V ducts	-circular bin with central duct plenum & perforated sidings
Heating Unit	direct or indirect fired using liquid or biomass fuel	-direct fired using kerosene -indirect fired using biomass furnace	indirect fired biomass furnace	direct oil-gas fired furnace
Capacity	0.25 tph and above	10 cavs/hr from 24-18% MC	1 tph up to 14% MC	0.7 tph minimum
Mode of operation/ Control	manual	manual	Electric control panel	Electric control panel
Blower Unit	electric or engine driven; axial or centrifugal fan	electric or engine driven centrifugal fan	electric driven centrifugal fan	electric driven centrifugal fan
Operating Cost, PHP	10.50	11.00	10.90	-

Table 6. Comparative performance of different milling process.

Milling Process	Head, %	Brokens. %	Total Head & Brokens, %
Hand Pounding	40.0	20.0	60.0
Steel Huller	46.5	16.9	63.4
Disk Huller	55.9	11.6	67.5
Rubber Rollers	62.0	8.0	70.0

Source: Tua, F.L. 1982. Paddy Deterioration in the Humid Tropics.

GASGA Seminar Document. German Agency for Tech. Cooperation (GTZ)

Table 7. Grade requirements for paddy.

Grading Factor \ Grade	Premium	1	2	3
M.C., % w.b.	14	14	14	14
Purity (min)	98.00	95.00	90.00	85.00
Foreign matters (max)	2.0	5.0	10.0	15.0
a). Weed Seeds & other crop seeds	0.10	0.10	0.25	0.50
b). Other Foreign Matters	1.90	4.90	9.75	14.50
Defective:				
Chalky & immature kernels (max)	2.00	5.00	10.00	15.00
Damaged Kernels (max)	0.25	1.00	3.00	5.00
Contrasting types (max)	3.00	6.00	10.00	18.00
Red Kernels (max)	1.00	3.00	5.00	10.00
Discolored kernels (max)	0.50	2.00	4.00	8.00

TRSD-SQCD No.1: 1980. Standard Specification For Palay.

National Food Authority, Quezon City, Philippines.

Table 8. Grade requirements for milled rice

Grading Factors \ Grade	Premium	1	2	3
Head Rice (min.%)	95.00	80.00	65.00	50.00
Big Broken (max.%)	3.00	10.00	10.00	20.00
Broken other than big broken (max.%)	1.90	9.75	24.00	29.00
Brewers (max.%)	0.10	0.25	0.50	1.00
Defectives (max. values,%)				
Damaged Kernels	-	0.25	0.50	2.00
Discolored	0.50	2.00	4.00	8.00
Chalky & immature	2.00	5.00	10.00	15.00
Contrasting types	3.00	6.00	10.00	18.00
Red Kernels	-	0.25	0.50	2.00
Red Streaked Kernels	1.00	3.00	5.00	10.00
Foreign Matters	-	0.10	0.20	0.50
Paddy per 100 grams	1	8	10	15
Moisture Content	14	14	14.00	14.00

TRSD-SQCD No. 1: 1980. Standard Specification For Milled Rice.  
National Food Authority, Quezon City, Philippines.

Table 9. Percentages of different products and by-products of corn milling.

Main Product			By-Product		
A	Grit No. 10	58%	A	Floured Corn	6%
B	Grit No. 12	11%	B	Bran	10%
C	Grit No. 14	3.5%	C	Germ	15%
		72.5%			31%

Source: NFA Standard for corn grits

Table 10. Daily generation of CO<sub>2</sub>, H<sub>2</sub>O, heat and dry matter loss of 1000 tons of paddy at different moisture contents

MOISTURE CONTENT, %	DRY MATTER TONS	CO <sub>2</sub> KG	H <sub>2</sub> O KG	HEAT KG	DRY MATTER LOSS, KG
13	870	4.26	1.738	45.38	2.897
14	860	10.32	4.22	110.06	2.031
15	850	28.152	11.485	299.72	19.142
16	840	68.769	28.056	732.014	46.763
18	820	230.492	54.041	2455.512	156.7348
20	800	618.4	252.307	6586.02	420.512

Source: Phil. Recommends for Rice Post-production Operations

Table 11. Daily generation of carbon dioxide, water, heat and dry matter loss of 1000 t of shelled corn at different moisture content.

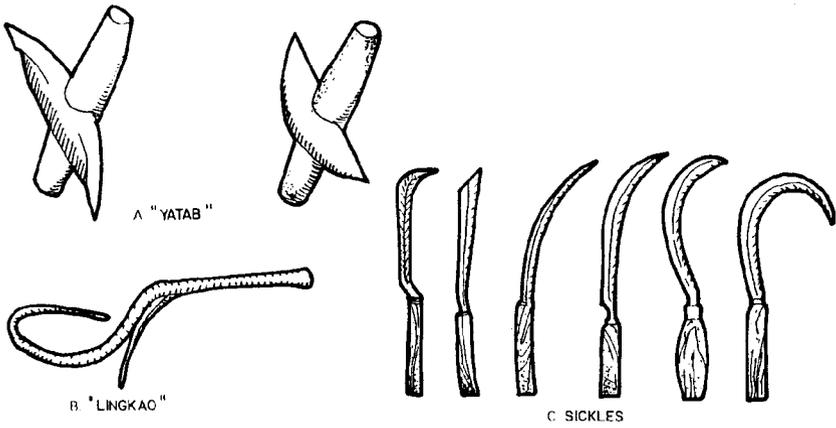
Moisture Content	Dry Matter (t)	CO <sub>2</sub> (kg)	H <sub>2</sub> O (kg)	Heat (kg)	Dry Matter Loss (kg)
13	870	16.22	6.64	172722	11.03
14	860	20.24	11.55	300800	19.2
15	850	52.47	21.47	558986	35.6
16	840	97.72	39.98	1041050	66.45
18	820	410.39	167.89	4372.093	279.07
20	800	1263.23	516.78	13457.666	959
22	700	2808.82	1149.06	29922323	1910
24	760	4238.23	17334.82	45151332	2882

Source: Philippine Recommends for Corn Post Production Operation, 1990

Table 12. Summary of government and private registered grain post harvest facilities. (as of Dec. 1991)

FACILITY	Government		Private		Total	
	No. tons	Cap.,	No. tons	Cap.,	No. tons	Cap.,
a. Rice Mill	65	217.4	13,374	7,667	13,439	7,885
b. Grader	29	115.0	-	-	29	115
c. Dehuller	8	24.0	-	-	8	24
d. Dryer	346	540.6	163	1,860	509	2,401
e. Polisher	5	25	-	-	5	25
f. Seed Cleaner	27	27	-	-	27	27
g. Sheller	34	36.1	567	666	601	702
h. Stack Conveyor	28	1,201.0	-	-	28	1,201
i. Thresher	25	16.9	1,679	1,376	1,704	1,392
j. Trucks	389	-	9,641 3,287,389		10,030 3,287,389*	
k. Barges	-	-	386	623,457	386 623,457*	
l. Warehouse	303	344,366.902	9,950 84,168,372		10,253 428,535,274*	
m. Corn Mill	-	-	1,761	846	1,761	856

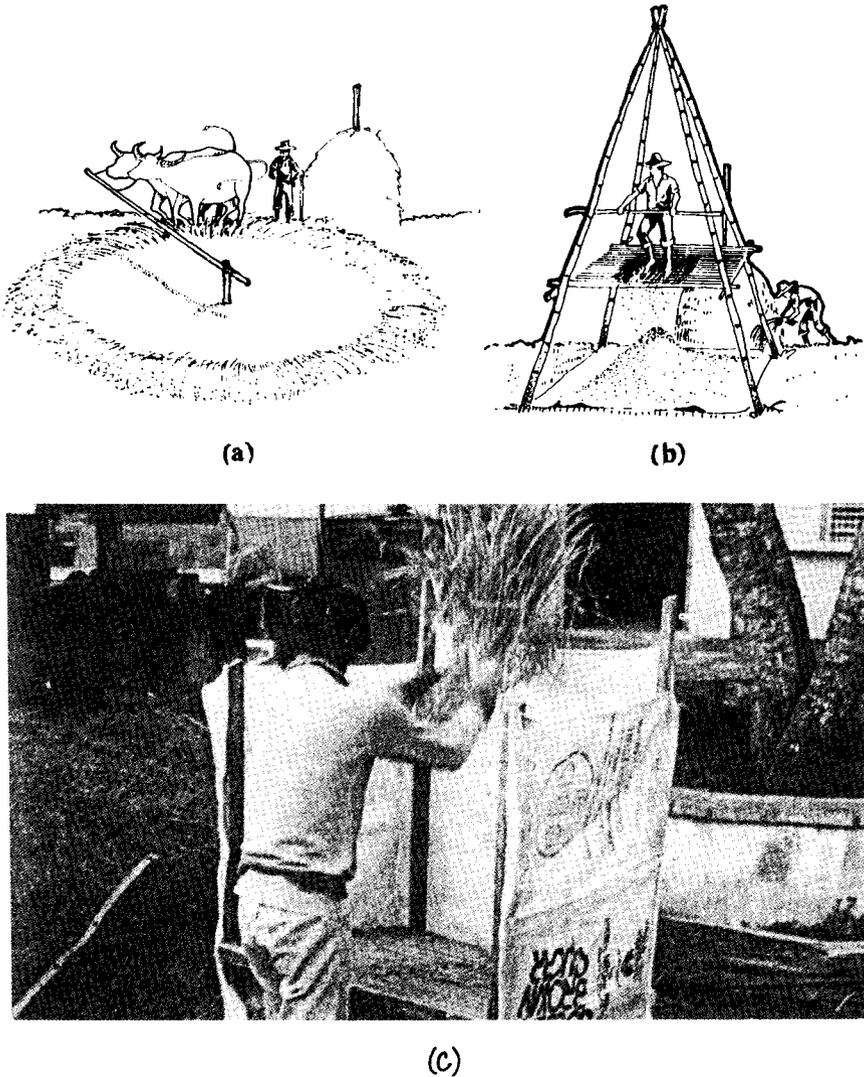
\* In 50 kg bag. Source: Business Regulation Directorate, NFA. Quezon City, Philippines



**Figure 1. Types of traditional tools used by farmers for rice harvesting.**  
Adapted from Phils. Recommends for Rice Post Production Operations. 1987



**Figure 2. Locally manufactured CAAMS-IRRI rice reaper.**  
Adapted from Phils Recommends for Rice Post Production Operations. 1987



**Figure 3. Traditional paddy threshing methods. a) Animal foot threading; b) Threshing with feet on an elevated platform; c) Manual threshing on threshing stand.**

Adapted from Phils Recommends for Rice Post Production Operations. 1897

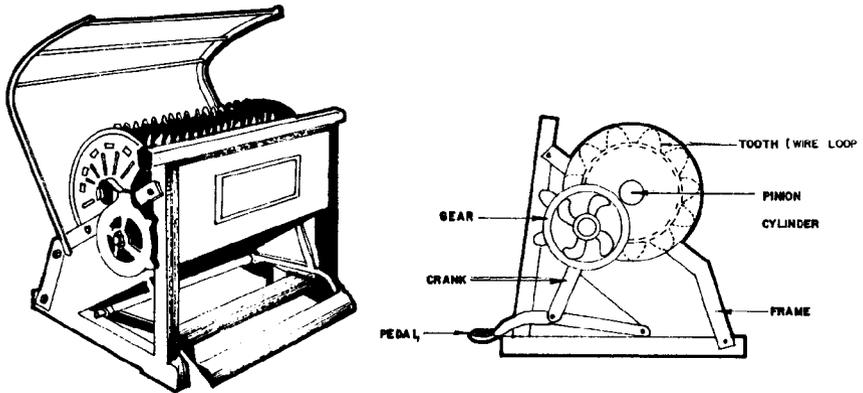


Figure 4. Gear type pedal rice thresher.  
Adapted from Torrizo F.M. Rice Production Manual. IRRI. 1983

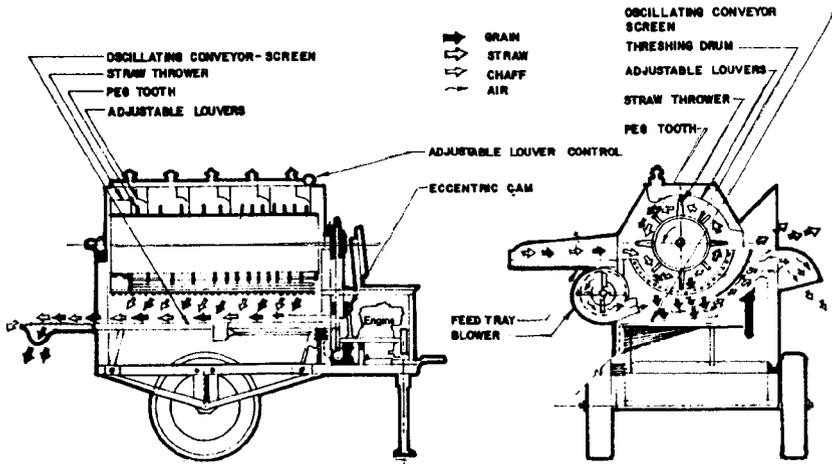
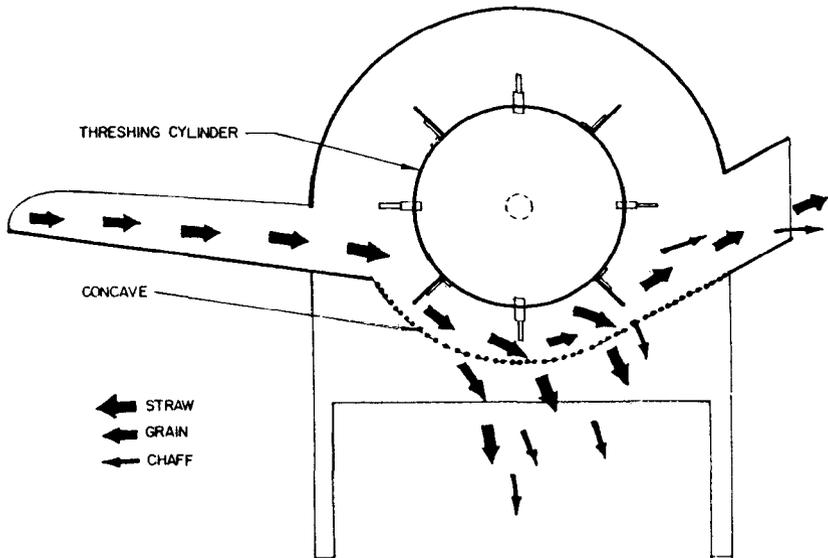
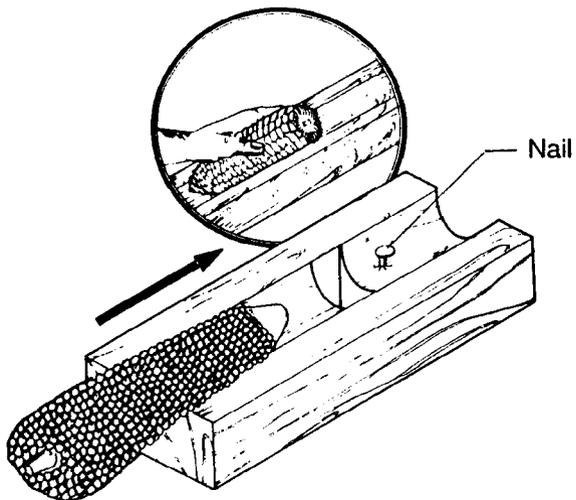


Figure 5. Portable or mobile axial-flow rice thresher.  
Adapted from Torrizo, F.M. Rice Production Manual. IRRI. 1983



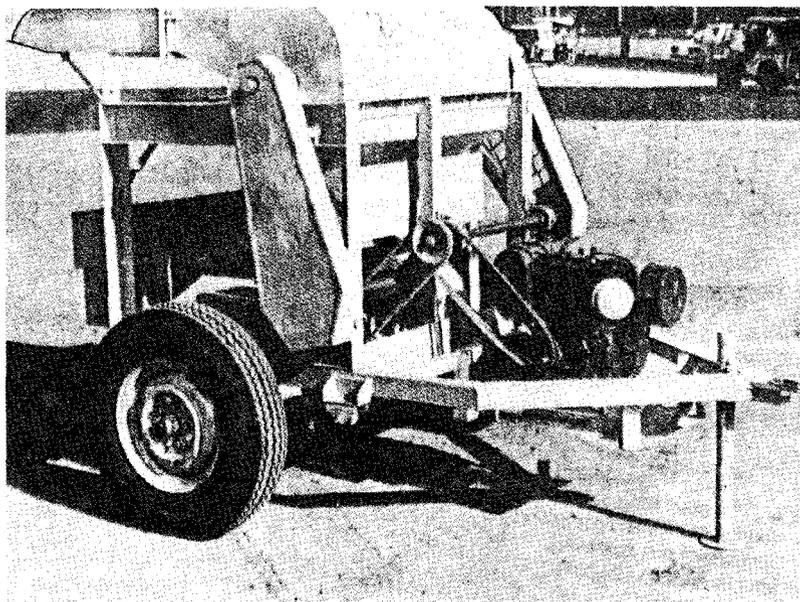
**Figure 6. Schematic diagram of a flow-thru rice tresher.**

Adapted from IRRI. Dept of Agriculture Engineering Semi-Annual Report. 1974

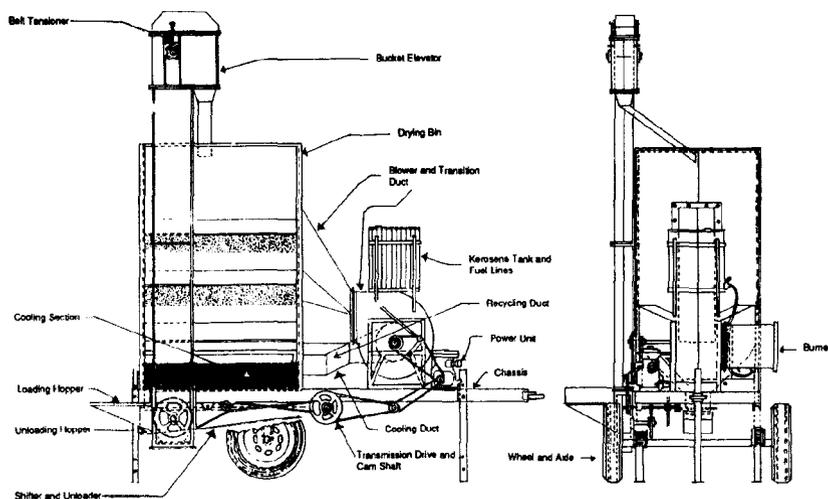


**Figure 7. "Gadgaran", a native wooden tool for manual corn shelling.**

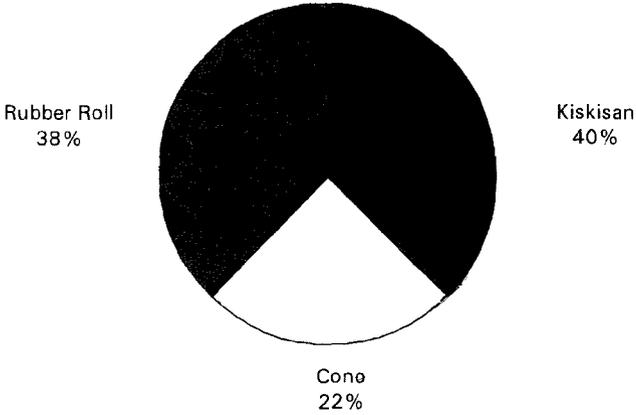
Adapted from Phils. Recommends for Corn Post Production Operations. 1990



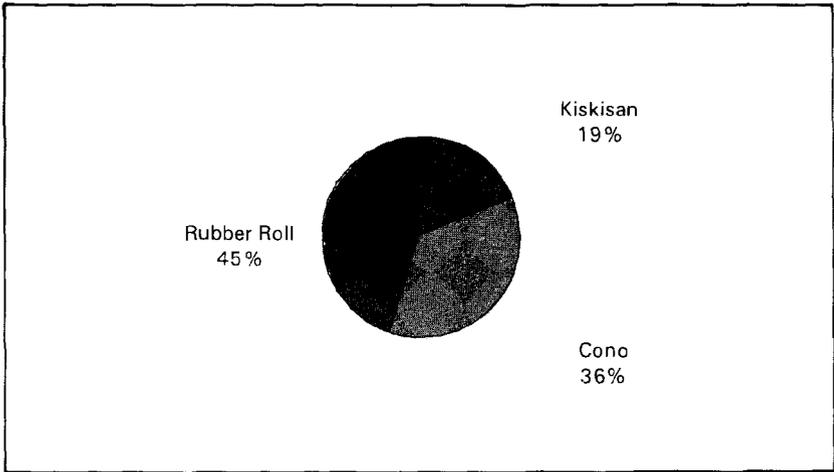
**Figure 8. NAPHIRE Improved Corn Sheller (NICS), a utility model.**  
Adapted from Manebog, E.S. and R.E. Manalabe. Development of an improved mobile maize sheller. 1986.



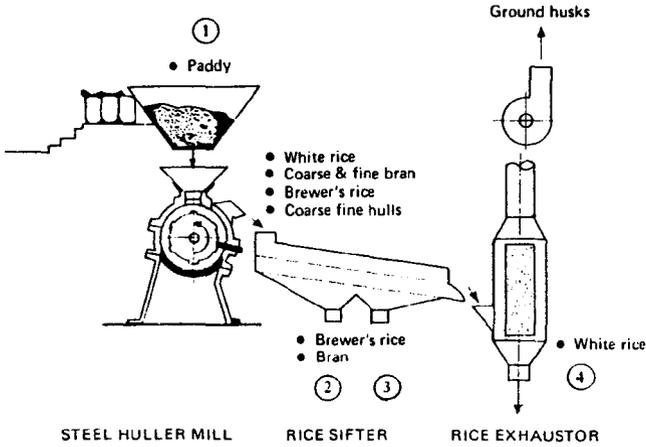
**Figure 9. A kerosene-fed mobile flash dryer.**  
Adapted from NAPHIRE. Operator's Manual. 1993



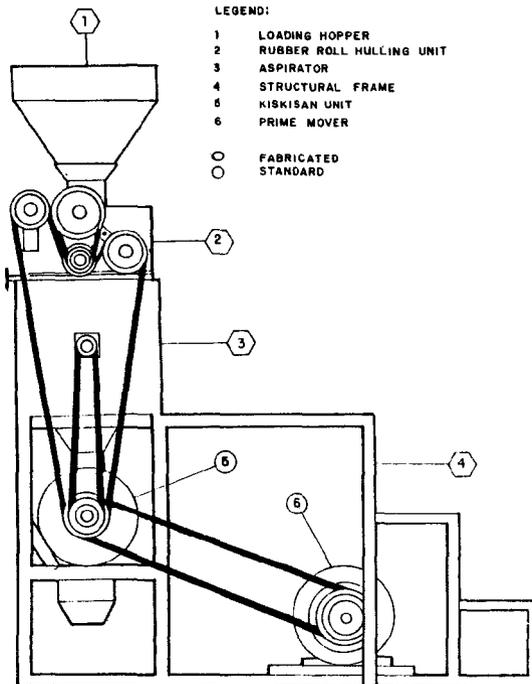
**Figure 10. Distribution of registered rice mills in the Philippines**  
Source: Directorate for Corporate Planning, NFA. 1991.



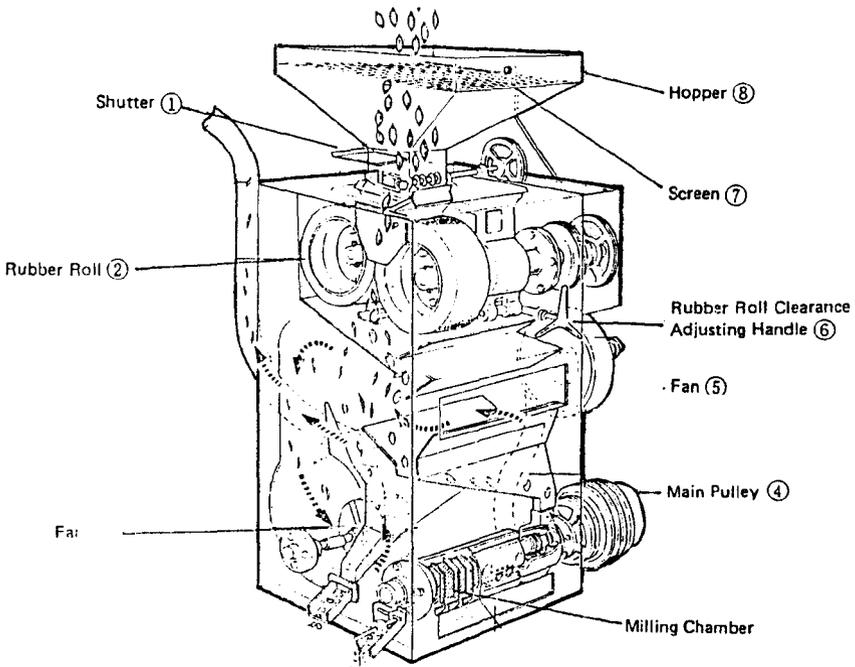
**Figure 11. Percentage input capacities of different rice mills.**  
Source: Directorate for Corporate Planning. NFA. 1991



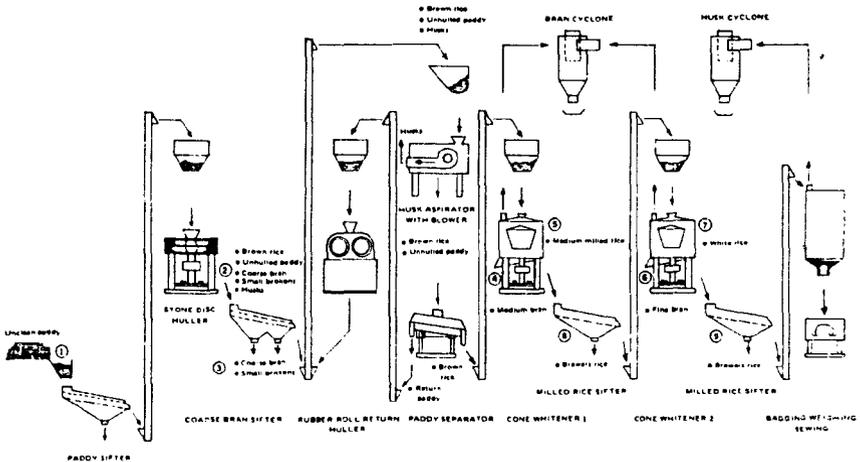
**Figure 12, Schematic diagram of "Kiskisan", steel huller single pass rice mill unit.**  
Adapted from Phils. Recommends for Rice Post Production Operations, 1987.



**Figure 13. Modified "Kiskisan", a combination of steel huller and rubber roll.**  
Adapted from Phils. Recommends for Rice Post Production Operations, 1987.



**Figure 14. Single pass rubber roll rice mill system.**  
Adapted from Mechaphil, Inc. Ricemaster Instruction Manual.



**Figure 15. Schematic layout of a "Cono" rice mill system.**  
Adapted from Phils. Recommends for Rice Post Production Operations. 1987.

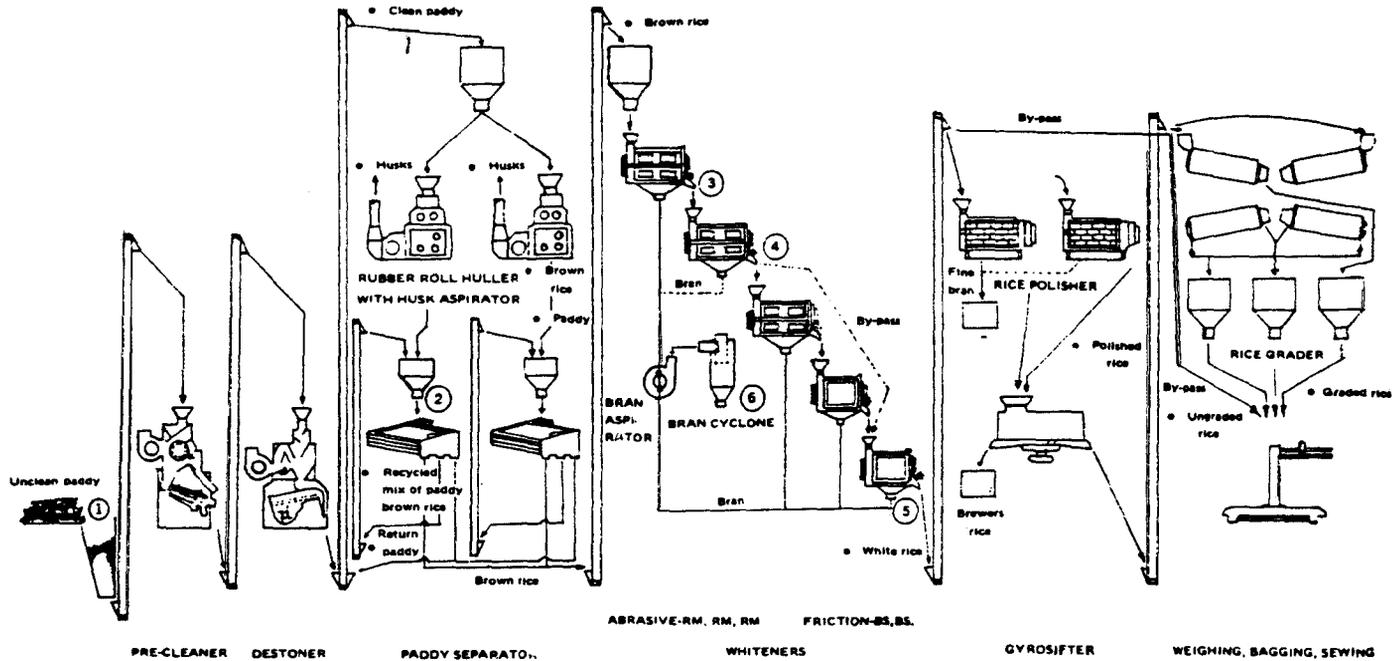
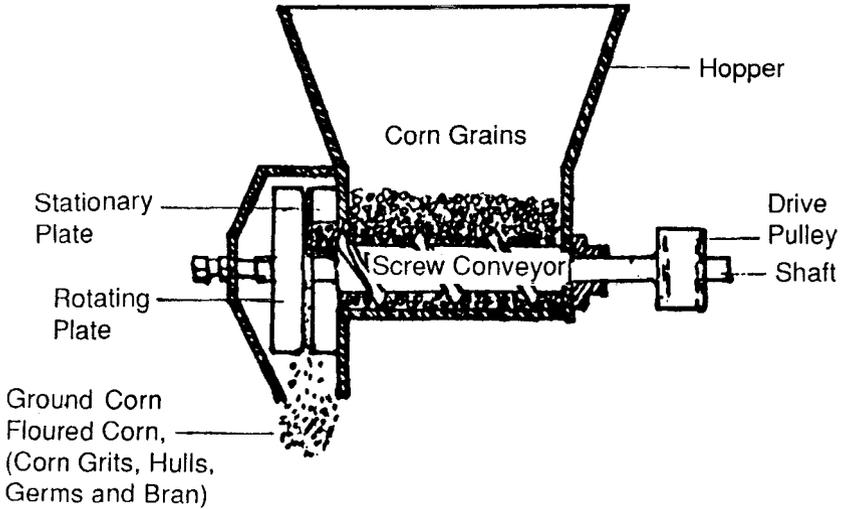
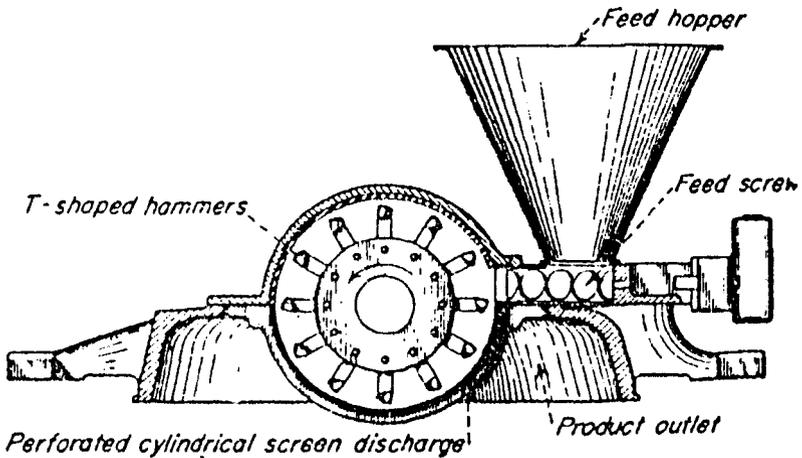


Figure 16. Schematic layout of a Japanese multipass rice mill system. Adapted from Phils. Recommends for Rice Post Production Operations. 1987



**Figure 17. Schematic diagram of a corn grinder.**  
Adapted from Phils. Recommends for Corn Post Production Operation. 1990.



**Figure 18. Schematic diagram of a hammer mill used in corn milling.**  
Adapted from Phils. Recommends for Corn Post Production Operations. 1990.



