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GALACTOMANNAN METABOLISM IN DEVELOPING NORMAL AND MAKAPUNO COCONUT ENDOSPERMS*

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ABSTRACT

The makapuno coconut endosperm accumulates 5 times more galactomannans than the normal during kernel development, which accounts primarily for its more viscous and gelatinous characteristics. On the other hand, the mature (11-12 mo) normal endosperm contains 3 times more of the mannan polymer. Mannose:galactose ratio of 3 for normal and makapuno galactomannans was observed at different stages of development. However, the intrinsic viscosity of purified galactomannans from mature normal endosperm was higher than that from makapuno (3.25 vs 2.20 dL/g) indicating longer chains for the former.

Three enzymes involved in galactomannan degradation were investigated in our laboratory: α-D-galactosidase, β-mannosidase and β-mannanase. α-D-galactosidase was observed to increase continually in maturing normal endosperm. In contrast, α-D-galactosidase was hardly detected in makapuno except in the mature stage where activity was 8,300-fold lower as compared to normal. Kinetic data indicated that the enzymes from normal and makapuno endosperms exhibit almost similar properties. α-D-galactosidase was inhibited strongly by D-galactose and to a lesser extent by myo-inositol, D-glucose-6-phosphate, L-arabinose and melibiose.

The activity of β-mannosidase decreased five-fold from 1.39 to 0.28 milliunit/mg protein in maturing normal endosperm while it increased nine-fold in water of the maturing nut (0.16 to 1.49 milliunit/mg). Similarly, makapuno β-mannosidase apparently decreased from 0.19 milliunits/mg protein in the 7-mo old nut to 0 in the 11-mo nut.

β-mannanase was detected in both normal and makapuno endosperms but to decrease during maturation. Its activity in makapuno was about two-fold higher than in the normal at several stages.

The possible role of these three enzymes in the formation of makapuno and in the germination of normal coconuts are discussed.

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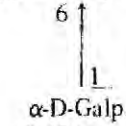
Introduction

The makapuno (mutant) coconut endosperm phenotypically differs from the normal in its characteristic soft and fluffy meat. In our previous paper (Mujer *et al.*, 1983), we found that a viscous, jelly-like precipitate could be obtained from both endosperms, but significantly in higher amounts in makapuno. The viscous component was identified to consist of galactose and mannose in a 1:3 ratio (Samonte *et al.*, 1985a) and corresponded to the galactomannan previously shown to be the major water-soluble polysaccharide in coconut (Balasubramaniam, 1976).

Galactomannans constitute 61% of the total polysaccharides in mature coconut kernel. It was suggested that they act as matrix polysaccharide and together with glucans, form the initial cell layers in developing coconut endosperm (Balasubramaniam, 1976). It is possible that some of the abnormal cellular properties of makapuno are direct consequences of an altered galactomannan metabolism, encompassing both biosynthesis and degradation. As part of our efforts to elucidate the abnormal cell growth of makapuno, we have worked on the characterization of the galactomannans of both normal and makapuno endosperms, and the three enzymes involved in their degradation: α-D-galactosidase, β-mannosidase and β-mannanase. This paper reviews the work we have done on these aspects.

Galactomannans

Plant galactomannans are reserve polysaccharides consisting of linear chains of (1→4) linked β-D-mannopyranosyl groups joined by (1→6)-linkages of α-D-galactose along the chain: $[\beta\text{-D-Manp-}(1\rightarrow4)]_n\text{-}\beta\text{-D-Manp-}(1\rightarrow4)$ –



Galactomannans vary in mannose: galactose (M/G) ratio, e.g. the galactomannan M/G from *Medicago sativa* L. (alfalfa, lucerne), 10-1.25; *Ceratonia siliqua* (carob, locust bean), 1.2-5.25; *Cyamopsis tetragonolaba* (guar), 1.3-7.0. Extensive lists are available in references Dey (1978) and Dea and Morrison (1975). Because of their chemical and physical properties galactomannans have found wide applications in industry, notably food, pharmaceuticals, cosmetics, paper products, paints and plasters, well-drilling and mining, and explosives and fire-fighting (Dey, 1978). In the food industry, galactomannans are used as thickening and gelling agents. The slow hydration of guar gum makes it a suitable base for delayed-release drugs. Galactomannans also imbibe water to form a mucilaginous paste that is impervious to water, thus, are used as a method of wetproofing in mining and manufacture of explosives and in increasing the wet strength of paper.

The coconut galactomannans isolated and purified by the method of Aspinall *et al.* (1953) (fresh and dry weight basis) were much higher in mature makapuno

endosperm than in normal (Table 1), although at 7-8 mo, the opposite was observed. These agree with our previous finding on viscous component precipitated by ammonium sulfate (Mujer *et al.*, 1983). Mannose: galactose ratio of 3 for normal and makapuno galactomannans was observed at different stages of development (Table 2), as compared to a ratio of 3.0, 2.57 and 2.0 as previously reported (Balasubramaniam, 1976; Kooiman, P., 1971; Rao *et al.*, 1961). Conditions in the purification of the crude galactomannans could account for the variation in ratios observed.

Table 1. Amount of galactomannans in normal and makapuno coconut endosperms

Age of Coconut+ (mo)	*Galactomannans (%)*			
	Normal		*Makapuno*	
	Fresh	*Dry*	*Fresh*	*Dry*
7-8	0.46	5.73	0.36	3.54
8-9	0.68	5.29	1.50	7.31
9-10	1.07	5.80	2.58	8.56
10-11	1.05	4.01	3.10	8.34

+After pollination
From Samonte *et al.*, 1985.

Table 2. Mannose: galactose ratio of normal and makapuno galactomannans at different stages of development [1]

Age (mo)	*Normal*	*Makapuno*
7-8	2.92 ± 0.06	2.94 ± 0.01
8-9	3.00 ± 0.03	2.93 ± 0.02
9-10	2.74 ± 0.02	2.72 ± 0.03
10-11	2.90 ± 0.04	2.99 ± 0.05

[1] Averages of two trials in 2 injections per trial. From Samonte *et al.*, 1985a.

The intrinsic viscosity of galactomannans from normal endosperm increased with age (from 1.05 to 3.25 dL/g) while that of makapuno increased from 8-9 mo to 9-10 mo but was similar in 9-10 to 10-11 mo samples (Table 3). This property of galactomannans from normal at 10-11 mo was higher than that from makapuno (3.25 vs 2.20 dL/g) indicating shorter and less coiled galactomannan polymers in makapuno than in normal. However since makapuno has much more galactomannan than normal, makapuno seems more viscous than normal.

To investigate further the polysaccharide composition of normal and makapuno endosperms, 8-9 and 10-11 mo old samples of both types of endosperms were subjected to a four-step fractionation for separating different types of galac-

Table 3. Intrinsic viscosities of purified galactomannans of normal and makapuno coconut endosperms

Age of Coconut+ *(mo)*	*Intrinsic viscosity (dL/g)* *Normal*	*Makapuno*
8-9	1.05	0.85
9-10	1.58	2.25
10-11	3.25	2.20

+After pollination
From Samonte *et al.*, 1985a

Table 4. Polysaccharide fractionation of 8 and 10 mo-old normal and makapuno endosperm

Fraction	*Polysaccharide (%)*+ *8 mo*		*10 mo*		*Type of poly saccharide* §
	Normal	*Makapuno*	*Normal*	*Makapuno*	
Hot-water (3)	3.77	6.97	1.64	11.10	
4% NaOH (3)	3.68 73	4.76 61‡	2.12	4.68 72	galacto-
17% NaOH (1)	1.00	1.80	1.89	0.89	mannan
(2)	0.45	5.02	1 30	3.02	
Hot 17% NaOH (1)	0.91 17	1.77	7.91	0.16 14	mannan
(2)	0.57	0.33	1.43	0.10	
Nonextractable Portion	1.18 10	1.45 7	0.42 3	3.34 14	cellu- lose
Total	11.56	22.10	16.71	23.47	

+Expressed as percent of polysaccharide in freeze-dried endosperm
§Based on results of Balasubramaniam (1976); these are being verified in our laboratory.
¶No. of extractions (in parentheses)
‡Percent of total polysaccharides
From Samonte *et al.*, 1985a.

tomannans according to a modified method of Balasubramaniam (1976). The hot water extract consists of water-soluble galactomannans. As the severity of extraction progresses, more mannan components are extracted. Table 4 shows that makapuno endosperm contained 2 and 8 times more water-soluble galactomannans than normal endosperm at 8 mo and 10 mo, respectively. The normal endosperms increased four-fold in its hot 17% NaOH fraction (mannans) while

makapuno decreased by 50% as the endosperm matured. The identity of the sugars in the different fractions is presently being determined by gas liquid chromatography to confirm these results. Also, the value of the non-extractable residue which corresponds to α-cellulase is being verified.

Enzymes Involved in Galactomannan Degradation

α-D-Galactosidase

α-D-Galactosidase catalyzes the hydrolysis of an α-D-galactopyranosyl linkage from an alkyl, aryl or a glycosyl (mono or oligo) residue or groups. In plants, especially in seeds where galactomannan is the reserve polysaccharide, one of enzyme's functions is to cleave α-D-galactosyl groups from α-D-galactose containing oligo- and polysaccharides. The resulting degradation products serve as a ready source of energy and cell metabolites (Dey, 1978).

In normal endosperm. α-D-galactosidase activity increased with age continually up and is inversely correlated with the amount of galactomannans at similar developmental stages of the normal endosperm (Table 5). The increasing trend of α-D-galactosidase activity also coincided with the increasing amounts of mannan polymer in the coconut endosperm as it matured. Thus, this enzyme has a major role in the accumulation of mannan in maturing normal coconut possibly as a consequence of galactomannan degradation. McCleary and Matheson (1975) reported on the depletion of galactomannans in the germinating seeds of lucerne, guar, carob and soybean which was accompanied by a rapid increase and then a decrease in α-D-galactosidase activity.

Table 5. α-D-galactosidase activity in normal coconut endosperms at various stages of development

Stage	*Age after pollination (mo)*	*Specific activity*[+] *(Units/mg protein)*
II	7-8	0.07[d]
III	8-9	0.23[d]
IV	9-10	0.59[c]
V	10-11	0.99[b]
VI	11-12	1.71[a]

[+]Average of six replications. Means followed by the same letter are not significantly different from each other at 0.05 level (Duncan's Multiple Range Test).

One unit of enzyme activity is defined as the amount tha hydrolyzes at one mole of *p*-nitrophenyl α-D-galactoside per min at 30°C and pH 7.5 under specified conditions.

From Mujer *et al.*, 1984a.

In makapuno endosperm. In contrast, α-D-galactosidase was not detected in the makapuno endosperm when the usual kinetic assay used for the normal samples was employed. However, activity was detected when the enzyme was incubated in the reaction mixture for 18-24 hr at 30°C. α-D-galactosidase activity was detected in the 11-12 mo old endosperm of makapuno but was 8300-fold lower as compared to the activity in the normal.

Properties of α-D-galactosidase. To understand better the regulation of α-D-galactosidase activity, the enzyme was isolated and partially purified employing ammonium sulfate fractionation (40-70% cut) followed by ion exchange chromatography on SP-Sephadex C50-120, and gel filtration on Sephadex G-200 and Sephadex G-100 (Mujer *et al.*, 1984b).

Two molecular forms of α-D-galactosidase termed A and B were separated on ion exchange column; both A and B were monomers with similar molecular weights of 23,000 and 26,000. Both α-D-galactosidase A and B exhibited optimum activity at pH 7.5. The partially purified A enzyme was stable over the pH range of 4-8 and had optimum activity at 50°C. Table 6 shows that the Km and Vmax values of the α-D-galactosidase from normal and makapuno are similar. Hence, the deficiency of activity in makapuno is not due to a possible mutation of the structural gene for α-D-galactosidase, but may be due to either a continuous repression of enzyme synthesis or the presence or absence of specific effectors.

The enzyme was inhibited strongly by D-galactose and to a lesser extent by myo-inositol, D-glucose-6-phosphate, L-arabinose and mellibiose (Table 7). It is possible that the presence of any of these sugars in excessive amounts could inhibit the activity in vivo. The hexose may also be formed from the α-D-galactosidase catalyzed hydrolysis of galactomannan. α-D-galactosidase was also inhibited by sulfhydryl specific reagents like iodoacetic acid which suggests that a sulfhydryl group participates in the enzyme catalysis.

Table 6. Kinetic properties of coconut α-D-galactosidase

Enzyme	*Km*	*Vmax*
Crude enzyme from normal	3.13×10^{-4} M	2.19×10^{-3} M
α-D-galactosidase A	3.46×10^{-4} M	1.38×10^{-3} M
Crude enzyme from makapuno (40-70% AS cut)	6.75×10^{-4} M	5.28×10^{-3} M

[+]Substrate is *p*-nitrophenyl α-D-galactoside.
Data from Mujer *et al.*, 1984[b]

Table 7. Inhibition of α-D-galactosidase A-catalyzed hydrolysis of *p*-nitrophenyl α-D-galactoside by sugars and related compounds at 30°C

Inhibitor	[*Inhibitor*] (*mM*)	*Inhibition* (%)
D-Arabinose	75	3
L-Arabinose	75	35
	50	25
	25	20
	5	8
L-Fucose	75	9
D-Galactose	5	88
	1	68
	0.5	46
	0.1	24
D-Glucose	75	5
D-Glucose-6-phosphate	75	60
	50	42
	25	22
	5	4
D-Mannose	75	3
	25	0
Myo-inositol	75	86
	50	80
	25	67
	5	26
Mellibiose	75	25
Sucrose	75	0
Coconut endosperm galactomannans	0.005%	5
	0.010%	7
	0.025%	24

From Mujer *et al.*, 1984b.

β-Mannosidase

β-mannosidase is an exohydrolase which catalyzes the removal of β-D (1→4)-linked D-mannosyl groups from the nonreducing end of their substrates, for example, D-manno-oligosaccharides and D-mannose-containing glycopeptides. The crude extract from the normal endosperm had optimal activity at pH 5.0 and 50°C. Using p-nitrophenyl-β-D-mannopyranoside as substrate, the specific activity of β-mannosidase was observed to decrease five-fold from 1.39 to 0.28 milliunit/mg in maturing normal endosperm while it increased nine-fold in the

water of the maturing nuts (0.16 to 1.49 milliunits/mg) (Table 8) (Samonte *et al.*, 1985b). On the other hand, the endosperm, the haustorium and the water of the germinating nut had very high activity, 10.39, 114.61 and 5.82 milliunits/mg, respectively. In the makapuno, β-mannosidase also decreased from 0.19 milliunits/mg protein to 0 in the 7-8 mo to 11-12 mo old endosperm.

The decrease in activity of β-mannosidase in both normal and makapuno indicate less amounts of sources of energy and cell metabolite were produced by the cell and would further indicate a slowing down of the mature cells' metabolism. In contrast, the high activity in the haustorium, endosperm and water of the germinating nut is reflective of the high metabolic activity the cells are in.

β-Mannanase

β-Mannanase is an endo enzyme which catalyzes the hydrolysis of β-D-(1→4)-mannopyranosyl linkages in mannans, galactomannans, glucomannans, galacto-

Table 8. β-Mannosidase in coconut during maturation and germination

Age after pollination (mo)	*Specific activity (milliunits/mg protein)*	
A. Endosperm	Normal	Makapuno
7-8	1.39	0.19
8-9	0.59	0.10
9-10	0.44	n.c.
10-11	0.26	n.c.
11-12	0.28	n.c.
B. Coconut water		
7-8	0.16	0.13
8-9	0.14	0.11
9-10	0.18	0.12
10-11	0.68	0.21
11-12	1.49	0.21
C. In germinating nut		
Endosperm	10.39	
Haustorium	114.61	
Water	5.82	

n.c., no color formed but high absorbance values obtained due to turbidity
n.d., not determined
One unit of enzyme activity is defined as the amount that hydrolyzes one μ mole of *p*-nitrophenyl-β- D-mannopyranoside per min at pH 5.0, 50°C, under specified conditions.
From Samonte *et al.*, 1985[b].

glucomannans and β-D-manno-oligopolysaccharides. The normal coconut β-mannanase in the crude extract was found to have optimum activity at pH 5.85. Although the enzyme still increased in activity at 50°C, the assays were done at 40°C. Takahashi (1983) noted a decline in thermal stability beyond 40°C with Actinomycetes β-D-mannanase.

β-D-mannanase activity was found to decrease in maturing endosperm of both normal and makapuno. It was also higher in makapuno than normal at several stages (Table 9) (De la Cruz, 1985). These results indicate an active degradation going on in both types of endosperms during its maturation. The higher mannanase activity in makapuno could account for its shorter and less viscous galactomannans. The increased levels of galactomannans could induce the higher activity of this enzyme in makapuno. McCleary and Matheson (1974) noted that β-D-mannanase activity parallels the levels of galactomannan during germination and suggested that the enzyme was instrumental in its breakdown.

Table 9. β-D-mannanase activity in normal and makapuno endosperms at various stages of development

Age after pollination	*Specific activity*[+] *(milliunits/mg protein)*	
	Makapuno	Normal
6-7	-	69.38 d
7-8	60.02 a	28.31 e
8-9	48.01 a	29.14 e
9-10	36.08 b	17.21 e
10-11	41.63 a	16.93 e
11-12	2.22 c	16.10 e

[+]Average of three replicates. Values followed by the same letter not significantly different by DMRT ($\alpha = .05$).

One unit of enzyme is defined as the amount that releases 1 μmole of reducing sugar expressed as glucose per min under specified conditions.

From Cruz, 1985.

Concluding Remarks

Galactomannan metabolism appears to have a major involvement in the formation of the makapuno phenotype. Galactomannans accumulate in maturing makapuno while in the normal, their level decreases with a parallel increase in the mannans. α-D-galactosidase plays a major role in this process. Its deficiency in

makapuno permits the accumulation of the viscous galactomannan while its high activity in the normal results in an increase in the water insoluble mannans.

On the other hand, β-mannanase and β-mannosidase were detected in both makapuno and normal. The higher levels of β-mannanase in the makapuno results in shorter forms of galactomannans of lower viscosity. Both enzymes had very low activity in the mature endosperm of both types although β-mannosidase activity had fallen to 0 at 9-10 mo. Perhaps these two enzymes are more important in germination. β-mannosidase exhibited a very high activity in the endosperm, haustorium and water of the germinating nut. The very low level of α-D-galactosidase and β-mannosidase in the makapuno might be a major factor in its nongermination. These enzymes are needed to break down the galactomannans to provide energy and metabolites for the seedling. Earlier reports indicate that the coconut seedling utilizes carbohydrates and not fat during the early stage of germination (up to 18 weeks) (Balasubramaniam *et al.*, 1973; Nathanael, 1969).

Our studies on the galactomannan metabolism of makapuno and normal coconut, more specifically on the enzymes involved in degradation, constitute only a small aspect of this very interesting phenomenon of makapuno. Further investigation is necessary to understand better the mechanism of and factors affecting the aberrant cellular formation of makapuno.

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Julian A. Banzon, Discussant

The makapuno has always been a very highly appreciated food. It is unique and rare. Why the coconut palm produces this unusual "nut" sometimes, and only sometimes, has been the object of much speculation. But there have been no answers to the question. Curiously also, the makapuno appears to be highly prized as food only in the Philippines. So the mystery of origin of the makapuno will have to be elucidated by Filipinos. The paper presented by Dr. Mendoza, which is actually a summary of several published papers by her research group headed by Dr. D. Ramirez, is "right on target" one may say.

There appears to be a parallel between fermentation and makapuno formation. In the course of alcoholic fermentation, acetaldehyde is formed and may be considered as the precursor of the ethanol as final product. But the acetaldehyde may be "fixed" as by addition of sulfites, in which case glycerol becomes the end product. Somewhat similarly, normal coconut endosperm formation, gives way to the "abnormal" soft endosperm characteristic of makapuno. As stated by the present author, "the abnormal cellular properties of the makapuno are direct consequences of the altered galactomannan metabolism. . ." It appears that the makapuno is the result of the inhibition of α-D-glucosidase activity.

If the biochemical process that results in makapuno formation can be elucidated, it would be a possibility to produce makapuno at will. This achievement will revitalize the coconut industry, which at present faces very stiff competition in the world's market. We desperately need new industrial products which can be produced from the coconut, products of many uses and more competitive. Galactomannans from makapuno, are such potential products. Galactomannans have a variety of industrial uses, not only in foods but also in such areas as textiles, paper, cosmetics, dyeing, pharmaceutical, etc.

We have yet a long way to go to make the makapuno an industrial crop; but it appears, we have a beachhead. We should be wise enough to recognize the situation and pour more effort – funds and minds into this beachhead.