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# CHROMATOGRAPHIC ANALYSIS OF CARBOHYDRATES IN COCONUT WATER

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## ABSTRACT

The water or liquid endosperm from newly opened mature coconuts was analyzed by high performance liquid chromatography (HPLC) using a silica-based micro-particulate cation exchange resin bed (Waters Sugar-PAK) column. Two samples of coconut water concentrate prepared by reverse osmosis at 20 and 30 bars pressure were also analyzed. Eleven HPLC elution peaks were observed corresponding to the following carbohydrates: glucose, fructose, sucrose, sorbitol, mannitol and oligosaccharides with degree of polymerization (DP) of 3, 4 5 and greater than 5. For coconut water from mature nuts the total concentration of sucrose, glucose and fructose was 30 g/l, that of galactose, xylose and mannose was 2.3 g/l, while the mannitol concentration was close to 0.3 g/l.

Gas chromatography (GC) was performed on aldononitrile and alditol acetate derivatives of standard and coconut water carbohydrates. Based on retention times the following sugars, which were not separately detected by HPLC, were observed but not quantitated by GC: arabinose, galactose, xylose and mannose.

## Introduction

Coconut water is an important by-product of the coconut industry with an annual Philippine production estimated to be in excess of $2 \times 10^9$ liters. It is the liquid endosperm of coconut and has been used to a limited extent as a beverage, especially from immature nuts, as well as fermentation substrate for the production of vinegar and 'nata de coco'. It is an excellent substrate for producing feed yeast (Caday *et al.*, 1980; Marfa *et al.*, 1982) and rhizobia inoculant (Mamaril and Aspiras, 1982). Efficient utilization schemes for coconut water require complete and definitive data on its chemical composition. Unfortunately, such data are not available and only limited information is available in the literature (Raghavan, 1976; Philippine Coconut Authority, 1979; Hagenmaier, 1980; Banzon and Velasco, 1982).

Two of the most practical and sensitive methods for carbohydrate analysis are high performance liquid chromatography (HPLC) and gas-liquid chroma-

tography (GLC). These chromatographic methods have been extensively developed since the pioneering work of Ramsey and Tswett early this century (McNair and Bonelli, 1967; Heftmann, 1975; Snyder and Kirkland, 1979). The development of rapid and automated HPLC methods, as well as highly selective columns, for saccharide analysis has been achieved within the last decade (Linden and Lawhead, 1975; Conrad and Palmer, 1976; Verhaar *et al.*, 1981). The use of such methods for coconut water analysis is facilitated by the availability of commercially available columns which can separate simple sugars and sugar alcohols (Waters Associates, 1982). These carbohydrates are the major components of coconut water. On the other hand, carbohydrate analysis by gas chromatography (GC) has been facilitated by rapid and practical procedure for derivatizing the carbohydrate components (Albersheim *et al.*, 1967; Crowell and Burnett, 1967; McGinnis, 1982).

The present paper deals with carbohydrate analysis using both liquid and gas chromatographic methods of fresh coconut water and of coconut water which had been concentrated by reverse osmosis. The experimental techniques are described for identifying and quantifying the carbohydrate components in coconut water. The HPLC results had been presented earlier (Bergonia *et al.*, 1984).

## Materials and Methods

A schematic flow sheet of methodologies for sample preparation and HPLC analysis is shown in Figure 1.

*Sample preparation.* Fresh coconut water samples from newly-opened mature coconuts were obtained from the local market or from a desiccated coconut factory. Concentrated coconut water was prepared by reverse osmosis (del Rosario, 1984) and ultrafiltered through XM300 membrane before HPLC analysis.

*HPLC Procedure.* The coconut water samples, which have been filtered as described above, were injected into a Waters Liquid Chromatographic System consisting of a Model U6K Universal Injector, a Model 6000A Solvent Delivery System and a Model 401 Differential Refractometer. A Sugar-PAK 1 column was used. This semi-preparative column contains a micro-particulate cation exchange resin bed consisting of 10um particles which are irregular in shape. It was designed to separate sugar alcohols and simple sugars up to oligosaccharides with degrees of polymerization equal to four. The temperature of the column was maintained at 90°C in a fabricated glass water jacket using a Haake Water Bath Model D3 with a recirculation pump.

The chromatograms were printed by an Omni-Scribe chart recorder at 0.25, 0.50 and 1.00 cm/min chart speeds. Attenuation was always set at 8x and the carrier solvent was usually pumped at 0.5 ml/min. Degassed and fresh doubly-glass-distilled water was used as carrier solvent. Also used as carrier in the later runs was 0.0001 M calcium acetate pH 6.8. Injected volumes usually ranged from 5 to 25 ul of liquid, depending on the con-

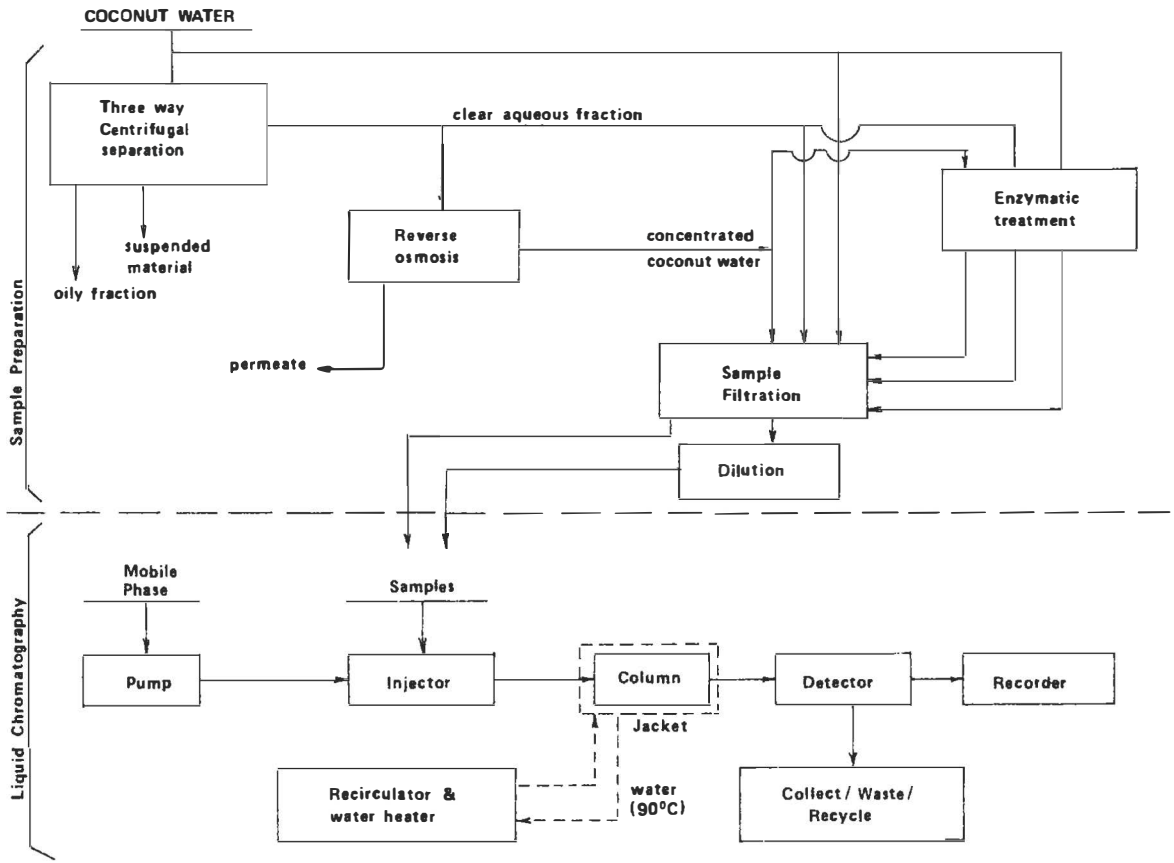


Figure 1. Flowsheet for Sample Preparation and HPLC Analysis.

centration of sugar in the samples. Back pressure ranged from 800 to 1300 psi, while elution time lasted from 12 to 15 minutes.

The peaks in the elution profiles were identified by "spiking" and quantitated using available standard sugars. Quantitation was based on peak height (Snyder and Kirkland, 1979). Mannitol was used as internal standard.

*Invertase treatment of coconut water.* To one milliliter of coconut water sample was added two milligrams of invertase powder (technical grade, Nutritional Biochemical Co., ICN Pharmaceuticals, Inc., Cleveland, Ohio, U.S.A.) suspended in one ml 0.04 M sodium acetate buffer at pH 5.0. The mixture was incubated at ca. 29°C for one hour and then heated in a boiling water bath for 20 min. The reacted samples were diluted with distilled water to ten times the original volume, filtered through Millipore 0.45um aqueous filter, and then chromatographed. For the control runs invertase suspensions were heated in a boiling water bath for 20 min before adding to coconut water samples.

*Amylase treatment of coconut water.* A commercial preparation of amyloglucosidase (Spiritamylase Novo 150L, 150 AGU/ml activity) was diluted 50 fold with 0.1M sodium acetate pH 4.8. A 2.0 ml aliquot of the diluted enzyme was added to the same volume of the solution of standard carbohydrate 5.0 mg/ml or coconut water sample, which had been diluted 1:1 with distilled water. For the control runs, the diluted enzyme was added to the same volume of the buffer. The mixtures were mixed well and allowed to stand for 45 minutes at ca. 30°C, and then placed in a boiling water bath for 20 minutes. The samples were cooled and then passed through a 0.45uM filter prior to HPLC analysis.

*Backflushing of HPLC column.* Backflushing was done by passing 100-150 ml of the carrier through the HPLC column in reverse direction whenever the back pressure exceeded 1500 psi. The back pressure was also reduced by not using the column for a few days. Sonic cleaning of the ends of the column greatly reduced the back pressure.

*Sample preparation for GC analysis.* A schematic diagram is presented in Figure 2 for sample treatment. Two types of carbohydrate derivatives were prepared for GC analysis, namely alditol acetates and aldo-nonitrile acetates.

Alditol acetate derivatives of coconut water carbohydrates and standard sugars were prepared using the method of Crowell and Burnett (1967) as modified by Manullang (1981). The coconut water sample (1.0 ml) was concentrated by vacuum evaporation in a rotary evaporator at 60°C prior to reduction of the component sugars to their corresponding alditols. The internal standard, 25 mg α-methyl-D-glucopyranoside, was added to the sample. Five milliliters of freshly prepared 0.5N $NH_4OH$ containing 20 mg $NaBH_4$ per ml was added to the resulting syrup. The mixture was allowed to stand at room temperature for 15 hours. Concentrated acetic acid diluted 1:1 with absolute methanol was then added dropwise until gas evolution


Figure 2. Flowsheet for Sample Preparation for GC Analysis.

stopped. This mixture was concentrated to a syrup as mentioned above in a rotary evaporator. The concentrate was then washed free of diborane by the addition of 5 to 10 ml methanol and then dried at 60°C in vacuum. This washing and drying was done five times and then the syrup was heated in an oven at 105°C for 10-15 minutes.

The sample was then acetylated by adding 7.5 ml acetic anhydride and 0.5 ml concentrated sulfuric acid. The reaction mixture was placed in a water bath shaker for one hour at 50-60°C, and then cooled. About 70 ml cold water was added. The derivatives were then extracted thrice with methylene chloride, using 25, 15, 10 ml portions successively, and evaporated to dryness at 75°C each time. One ml water was added to the extract and also evaporated to dryness. Lastly, the derivatives were dissolved in 2.0 ml acetic anhydride prior to injection into the gas chromatograph.

For the standards, 5-, 10-, and 15- and 20-mg portions of arabinose, galactose, glucose, mannose and xylose were weighed in separate containers, such that one mixture contained equal amounts of each of the five standards. The resulting mixtures were treated as above in place of the coconut water syrup, including the addition of α-methyl-D-glucopyranoside as internal standard.

The derivatization procedure of McGinnis (1982) for preparing the aldononitrile acetates was followed with minor modifications. About 1 ml coconut water concentrate was lyophilized and then suspended in 1.0 ml pyridine containing 2.5% w/v hydroxylamine hydrochloride and 0.5% w/v α-methyl-D-glucopyranoside. The derivatizing mixture was heated in an oven for 45 minutes and cooled. Then 0.4 ml acetic anhydride was added and the resulting mixture heated for another hour and cooled. The mixture was evaporated to dryness by bubbling with $N_2$ gas. Washing was done using 1.0 ml each of 3N HCl, 0.5M $NaHCO_3$, $H_2O$ and $H_2O$ respectively, each time discarding the aqueous layer. Lastly, the derivatives were then taken up in $CHCl_3$ which had been previously dried over $Na_2SO_4$.

*Gas chromatography.* A Shimadzu GC-3BF gas chromatograph with FID detector was used for both alditol acetate and aldononitrile acetate derivatives. The column packing was 3% OV-225, injection temperature was 225°C and the column temperature was maintained constant at 200°C. The air and hydrogen pressures were 1.0 and 0.9 kg/cm$^2$, respectively. Nitrogen pressure was set at 0.8 and 1.4 kg/cm$^2$ for the alditol and aldononitrile acetates, respectively. The chromatograph sensitivity was set at 10, with range setting of 4. The chart speed was 2.5 mm/minute. For quantification, peak heights were compared to the α-methyl-D-glucopyranoside (5 mg/ml) internal standard.

## Results and Discussion

*HPLC Elution profiles*

The high performance liquid chromatogram of fresh coconut water is presented in Figure 3a while that of coconut water, which had been concentrated by reverse osmosis and diluted tenfold prior to analysis. is shown

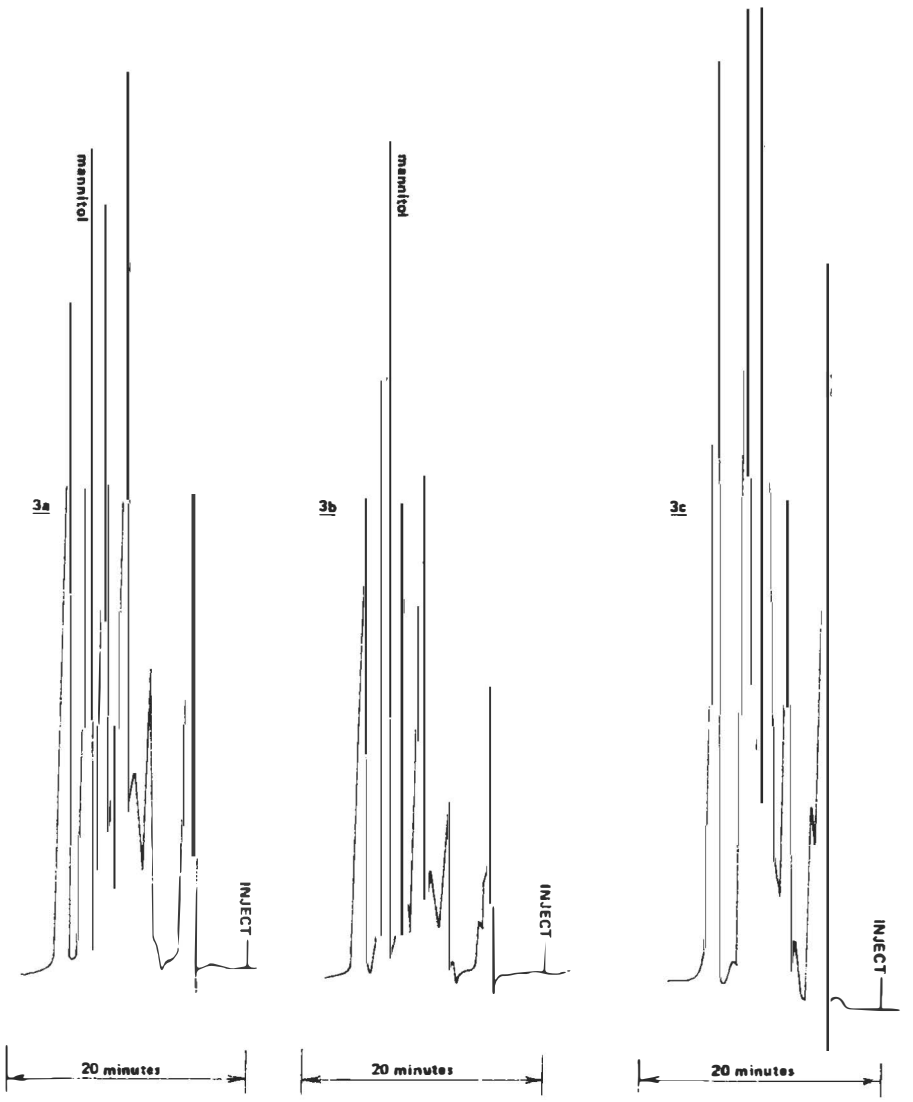


Figure 3. HPLChromatograms of 5μl ultrafiltered coconut water (3a), 10μl diluted coconut water concentrate (3b), and 3μl coconut water (3c). Samples in Figures a and b also contained 50μg mannitol as internal standard

in Figure 3b. The corresponding HPLC pattern for concentrated and undiluted coconut water at the same chart speed, i.e. 0.25 cm/min, is presented in Figure 3c. To the samples in Figures 3a and 3b, but not to the sample of Figure 3c, was added mannitol as internal standard. The elution profile of

concentrated and diluted coconut water without added mannitol is shown in Figure 4. The elution patterns of the different coconut water samples showed eight major and three minor peaks which are numbered in the order of increasing retention time.

*Identification of elution peaks*

Table 1 lists the retention times of the HPLC elution peaks relative to peak no. 7. The retention time was also calculated for each "spiked" peak or the peak that increased in height after adding to the sample a known carbohydrate standard. This table shows that peak 3 is probably maltopentaose. Peaks 4 and 5, which are quite small and have wide spreads of retention times, are more difficult to assign but could correspond to maltotetraose and maltotriose. Peak 6 corresponds to sucrose, maltose or cellobiose, peak 7 is glucose while peak 8 could be mannose, galactose or xylose. Peak 9 is fructose, peak 10 is mannitol and peak 11 is sorbitol. Peaks 1 and 2 could correspond to oligosaccharides with degrees of polymerization (DP) greater than five.

An interesting broadening/splitting phenomenon for peak 6 was observed during the course of the HPLC experiments. Early HPLC profiles of coconut water showed a single peak 6 which was cleanly separated at the baseline from peak 7 (Figure 5a). Later profiles showed merging of peaks 6 and 7, i.e. reduced degree of resolution, accompanied by reduced peak 6 height and increased heights for peaks 7 (glucose) and 9 (fructose) as shown in Figures 4 and 5b. Further runs showed a pronounced broadening/splitting of peak 6 as presented in Figures 3a and 3b.

The same broadening/splitting phenomen was observed for two reagent grade samples (Baker and Mallinckrodt) of sucrose which were used as spiking standards. For example, Figure 5b is the HPLC profile of reagent grade sucrose taken after the Sugar-PAK column had been used many times. Figures 6a and 6b correspond to sucrose spiked with fructose and glucose, respectively. The figures indicate that the broadened/split peak for sucrose also contains invert sugar, i.e. glucose and fructose. Therefore, the broadening/splitting phenomenon observed for pure sucrose could be explained by sucrose inversion in the HPLC column. In fact, the manufacturer has cautioned the HPLC user about this problem and suggested methods for solving this (Waters Associates, 1982).

The identification of peak 6 was done with the aid of invertase (technical grade powder) whose elution profile is shown in Figure 7a (with added mannitol). A band was observed with the same retention time, within experimental error, as peak 6. This band cannot correspond to sucrose (which should have been hydrolyzed by the enzyme) and is probably a stabilizing agent. The elution pattern of fresh coconut water with added active invertase is presented in Figure 7b. The corresponding pattern with added inactive invertase is shown in Figure 7c. It is clearly seen in Figures 7a, b and c that

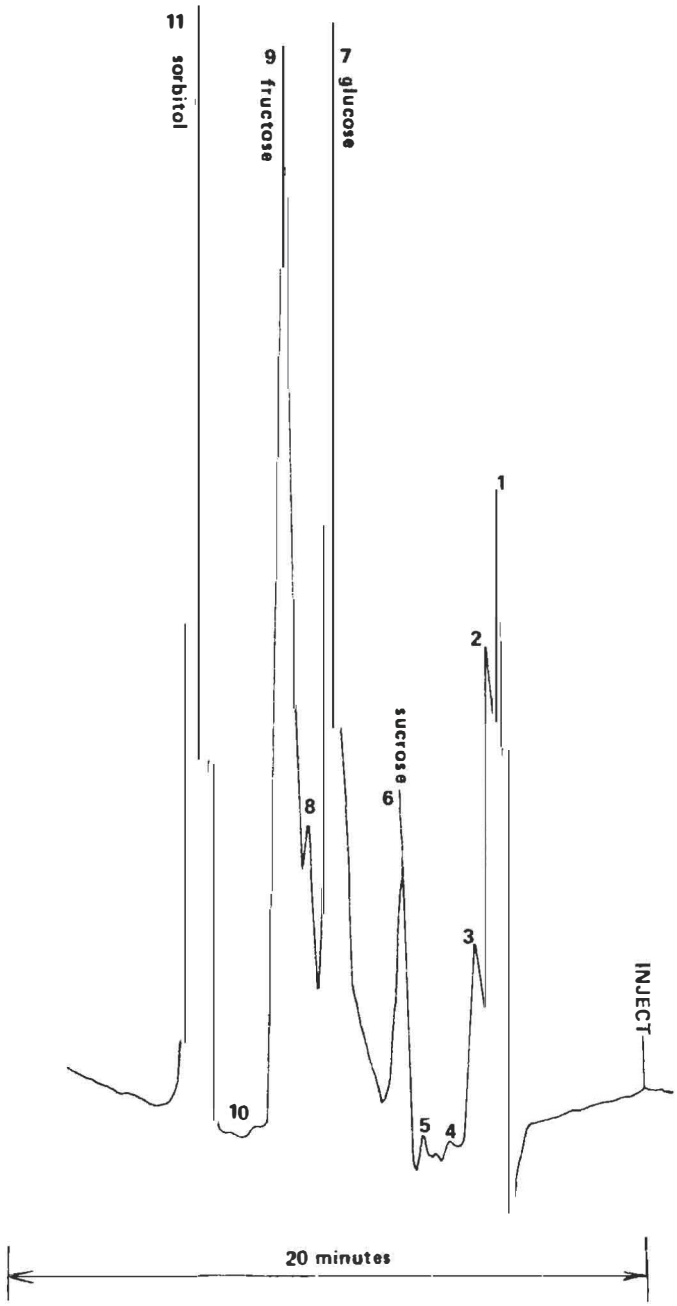


Figure 4. HPLC elution profile of 10$\mu$l diluted (10x) coconut water concentrate in carrier water.

Table 1. Relative HPLC Retention Times of Standard and Detected Carbohydrates in Concentrated Coconut Water.

| *Standard/Peak No.* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *10* | *11* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| None* | 0.49 | – | – | – | – | 0.78 | 1.00 | 1.09 | 1.14 | – | 1.41 |
| Maltose | 0.49 | 0.52 | 0.54 | – | – | 0.78* | 1.00 | 1.09 | 1.14 | – | 1.41 |
| Cellobiose | 0.49 | 0.52 | 0.55 | – | – | 0.78* | 1.00 | 1.09 | 1.14 | – | 1.41 |
| Sucrose | 0.49 | 0.51 | 0.54 | – | – | 0.78* | 1.00 | 1.10 | 1.14 | – | 1.42 |
| Glucose | 0.49 | 0.52 | 0.55 | – | – | 0.78 | 1.00* | 1.10 | 1.14 | – | 1.41 |
| Fructose | 0.49 | 0.51 | 0.54 | – | – | 0.78 | 1.00 | 1.09 | 1.14* | – | 1.41 |
| Xylose | 0.49 | 0.52 | 0.55 | – | – | 0.78 | 1.00 | 1.09* | – | – | 1.41 |
| Galactose | 0.49 | 0.52 | 0.54 | – | – | 0.79 | 1.00 | 1.08* | – | – | 1.41 |
| Inositol | 0.50 | 0.52 | 0.55 | – | – | 0.79 | 1.00 | 1.09 | 1.11 | 1.20* | 1.39 |
| Mannitol | 0.49 | 0.52 | – | – | – | 0.79 | 1.00 | 1.09 | 1.14 | 1.25* | 1.42 |
| Sorbitol | 0.49 | 0.52 | 0.55 | – | – | 0.78 | 1.00 | 1.09 | 1.14 | – | 1.41* |
| None | – | – | 0.56 | 0.63 | 0.71 | 0.78 | 1.00 | 1.09 | 1.14 | 1.26 | 1.42 |
| Maltopentaose | – | – | 0.56* | – | 0.69 | 0.78 | 1.00 | 1.08 | 1.14 | 1.26 | 1.42 |
| Maltotetraose | – | – | 0.56 | 0.58* | 0.71 | 0.76 | 1.00 | 1.06 | 1.12 | – | 1.40 |
| Maltotriose | – | – | 0.55 | 0.61 | 0.66* | 0.77 | 1.00 | 1.08 | 1.14 | 1.25 | 1.41 |
| Mannitol | – | – | 0.58 | 0.66 | 0.73 | 0.79 | 1.00 | 1.10 | 1.14 | 1.26* | 1.44 |
| None | – | – | 0.49 | 0.59 | 0.68 | 0.75 | 1.00 | 1.04 | 1.13 | 1.20 | 1.41 |
| Mannose | – | – | – | 0.60 | 0.70 | 0.78 | 1.00 | 1.08* | 1.14 | 1.25 | 1.43 |
| Xylose | – | – | 0.51 | – | 0.71 | 0.76 | 1.00 | 1.07* | 1.15 | 1.23 | 1.42 |
| Arabinose | 0.43 | 0.44 | 0.49 | – | – | 0.80 | 1.00 | 1.08 | 1.14<br>1.19* | 1.29 | 1.47 |

*Concentrated coconut water with no added standard carbohydrate.

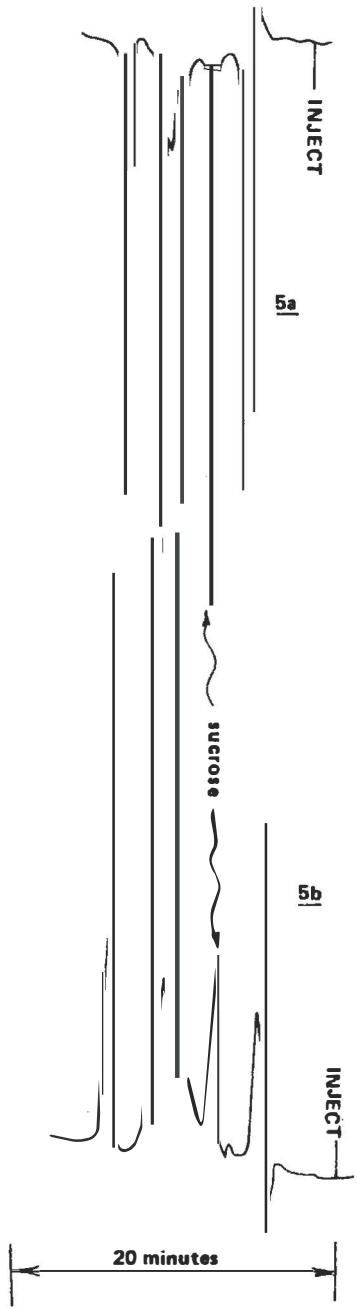


Figure 5. Elution profiles of 10μl diluted (10x) coconut water concentrate (early run – 5a; later run – 5b) with water as mobile phase.

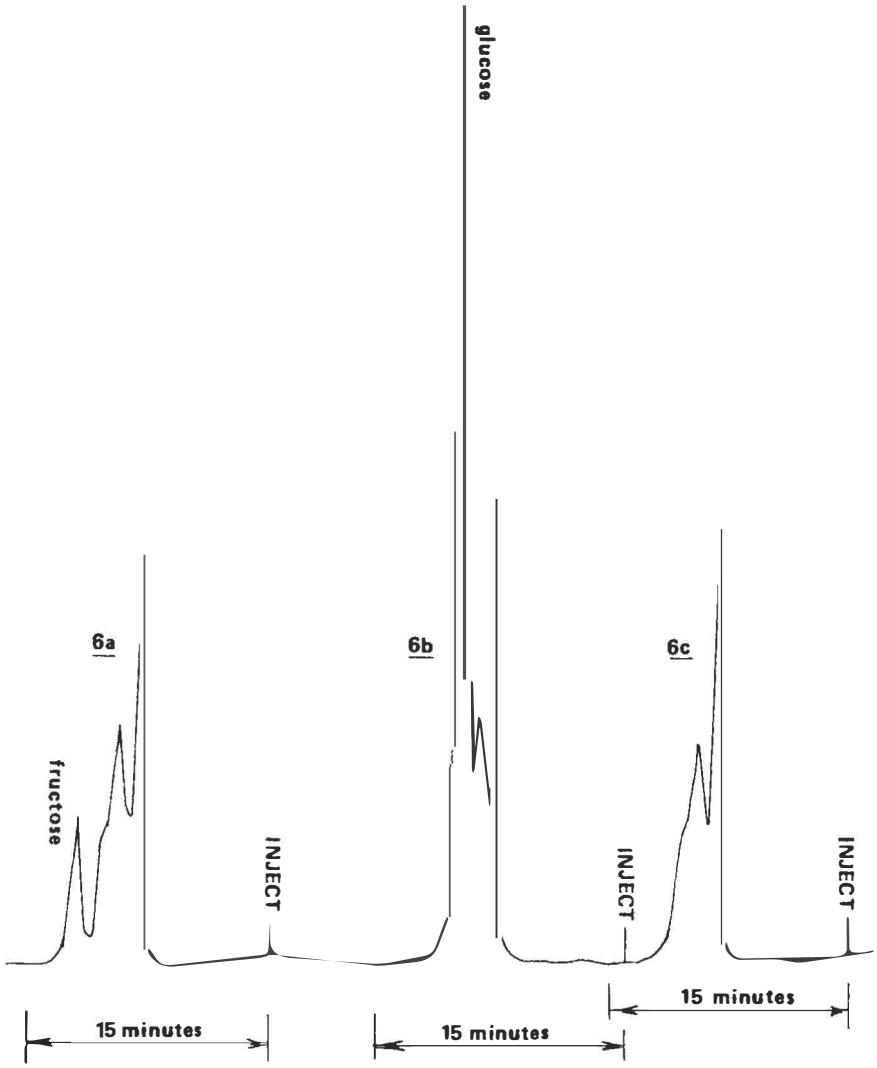


Figure 6. "Spiking" of 50μg sucrose samples with 10μg fructose (6a) and 40μg glucose (6b). Figure 6c is the HPLChromatogram of sucrose alone. The carrier used was 0.0001M calcium acetate.

with active enzyme, peak 6 of the original coconut water disappeared and only the residual band characteristic of the enzyme preparation remained. At the same time, the peak heights increased for peaks 7 and 9, which correspond to glucose and fructose, respectively. A control experiment was done wherein invertase was added, together with sorbitol as internal standard, to

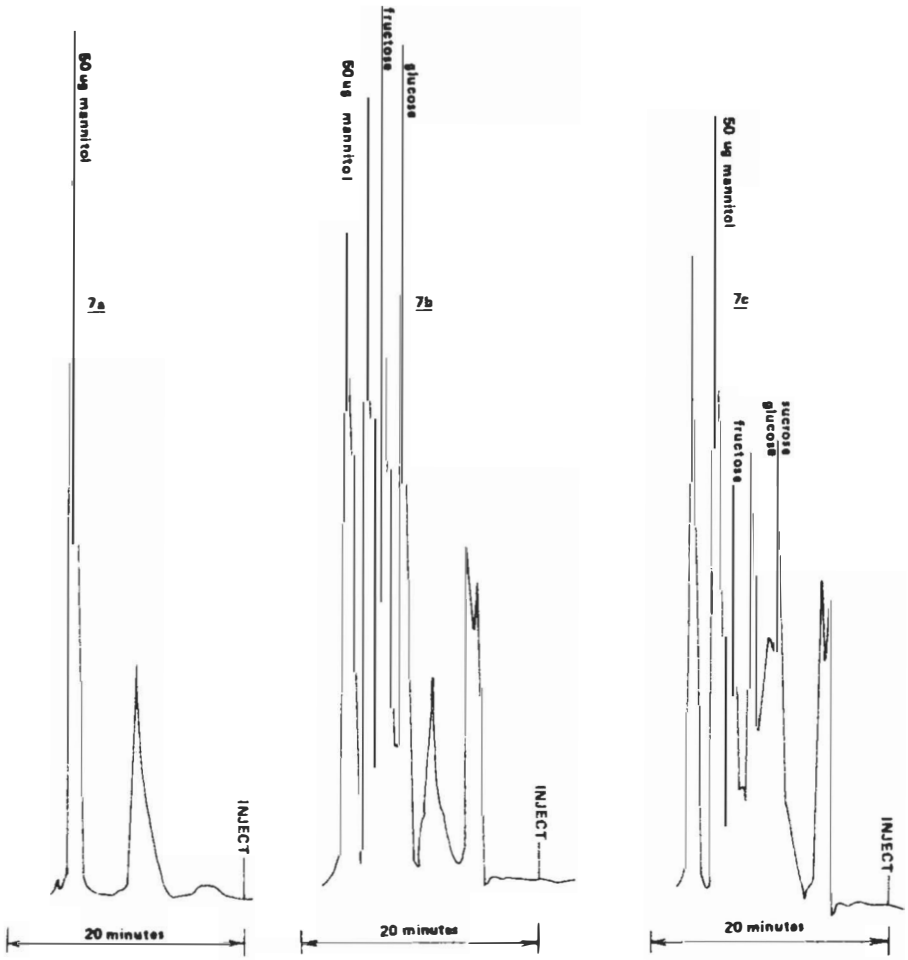


Figure 7. Elution profiles of invertase (7a), coconut water with active invertase (7b), and inactive invertase (7c) with mannitol as internal standard.

solution of maltose (Figures 8a and 8b) and sucrose (Figures 8c and 8d). The enzyme did not hydrolyze maltose, as expected, and did not change its HPLC profile. However, sucrose was hydrolyzed by the enzyme and its elution profile was drastically altered. This qualitative conclusion can be made inspite of complicating factors due to on-column hydrolysis of sucrose and the residual elution band of the invertase preparation. Reducing sugar analysis (using dinitrosalicylic acid; Miller, 1959) of the sucrose and maltose solutions, which had been treated with active invertase, showed a quantitative increase in reducing equivalents for sucrose but not for maltose. Both

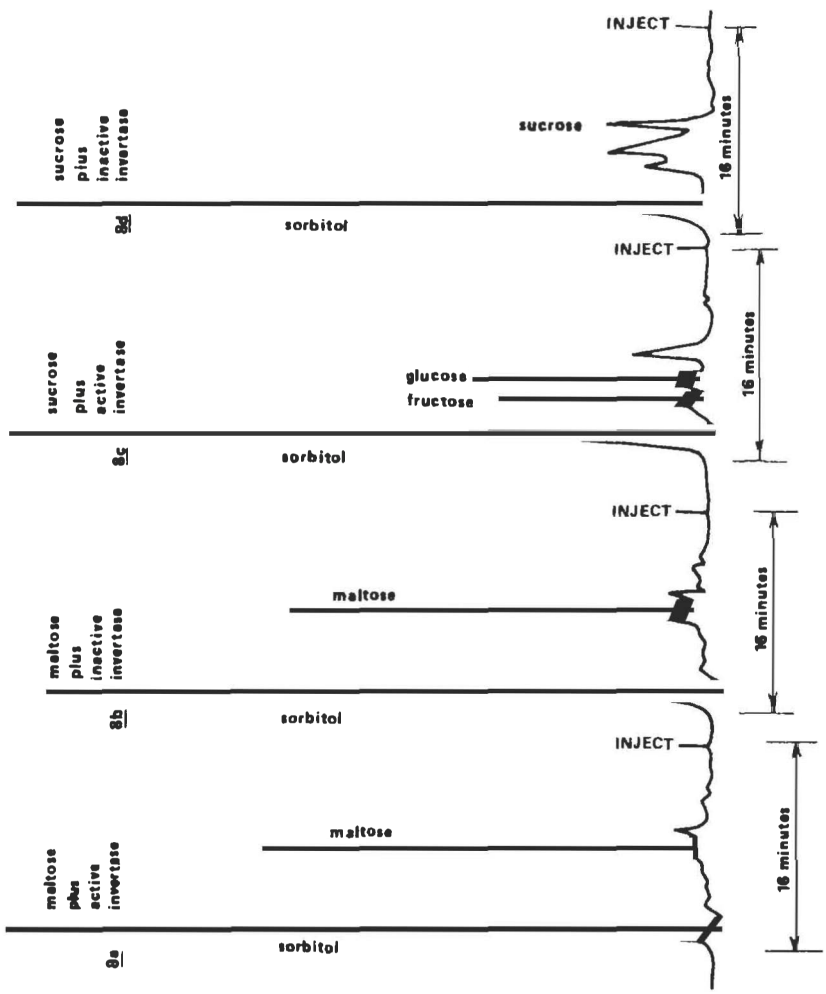


Figure 8. HPL Chromatograms of 10-$\mu$l samples of 4 mg/ml sugar and 0.1 mg/ml invertase in 0.005M acetate buffer pH 5.0 plus 50$\mu$g sorbitol as internal standard. The mobile carrier was 0.0001M calcium acetate.

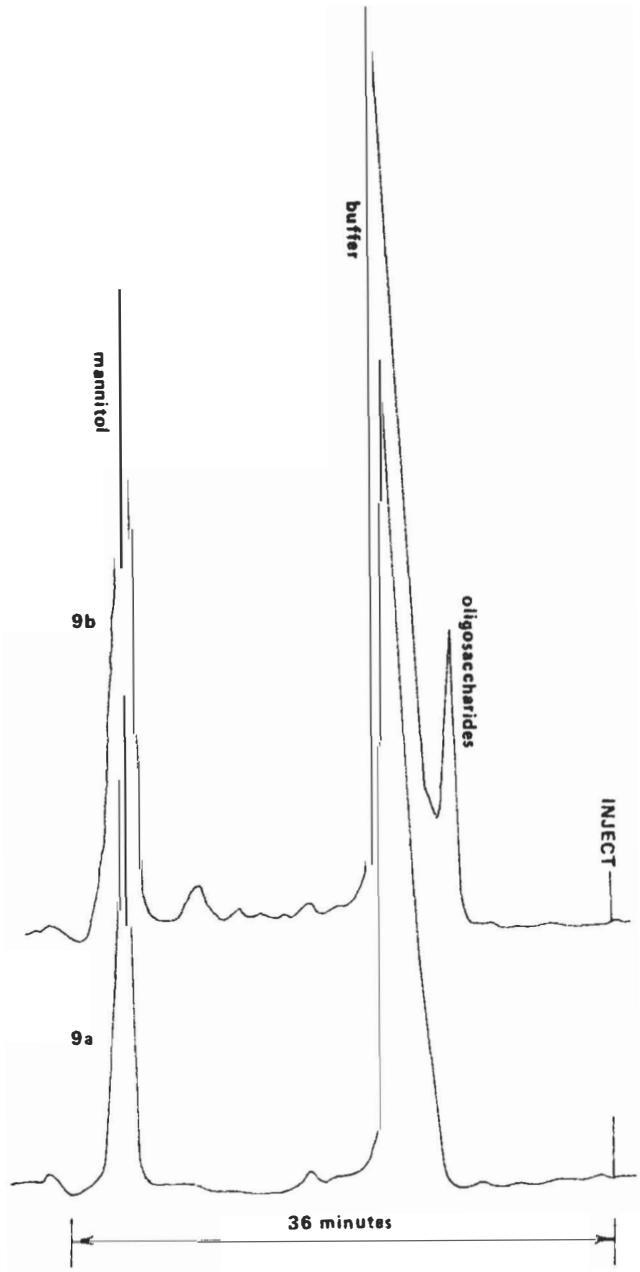


Figure 9. HPLChromatograms of 10μl each of 0.1M pH 4.8 sodium acetate buffer blank (9a) and amylase in the acetate buffer (9b), both with 30μg mannitol standard.

results of HPLC and reducing sugar determination show conclusively that peak 6 corresponds to sucrose and not maltose. The possibility of peak 6 being cellobiose is highly unlikely on the basis of the results of the above-mentioned treatment with invertase.

Amylase treatment of the samples was studied in terms of changes in the HPLChromatograms. Figure 9 shows that sodium acetate has a prominent HPLC peak while the commercial amylase preparation (Novo) also contained dextrins (DP > 5) and small amounts of monosaccharides. The action of the enzyme on starch is shown in Figure 10 where the emergence of a prominent glucose peak is seen after enzymatic treatment. After incubating maltose with the enzyme the HPLC peak increased its retention time from 0.61 to 0.77; this corresponds to the disappearance of the original maltose peak and the appearance of a glucose peak (Fig. 11). This shows the complete enzymatic hydrolysis of maltose to glucose.

After amylase treatment of coconut water, the glucose peak showed a significant increase in height. This increase in glucose concentration could only come from the hydrolysis of sucrose or oligosaccharides (maltose is absent as shown earlier). The sucrose peak height did not show a significant change as shown in Figure 12. Unfortunately, changes in the oligosaccharide peak height were masked by the presence of this peak in the enzyme preparation. Therefore, the conclusion that maltosaccharide peaks are present in coconut water derives partial but not definitive support from the increase in the glucose peak height after amylase treatment as well as from the expected retention times for such molecules as previously shown by the equipment and column manufacturer (Waters Associates). However, the presence of maltosaccharides in coconut water is *not* supportive of the previous findings of Balasubramiam (1976) that the polysaccharides of mature coconut kernel consists of 61% galactomannan, 26% mannan and 13% cellulose. Needless to say, further research should be done in order to positively identify the HPLC oligosaccharide peaks.

Figure 13 shows HPLChromatograms of coconut water concentrates prepared by reverse osmosis at 30 and 20 bars pressure. The concentrates were prepared several months apart from mature coconuts purchased by the desiccated coconut factory. The close similarity of the chromatograms shows that the same major carbohydrate components are present in coconut water from mature nuts.

*Results of gas chromatography*

The gas chromatograms of the aldononitrile and alditol acetate derivatives of coconut water are presented in Figure 14(a) and (b), respectively. The use of pyridine as acetylation catalyst for the first type of derivatives resulted in severe solvent peak tailing which precluded accurate quantitation of the carbohydrate components. However, several peaks may be clearly seen

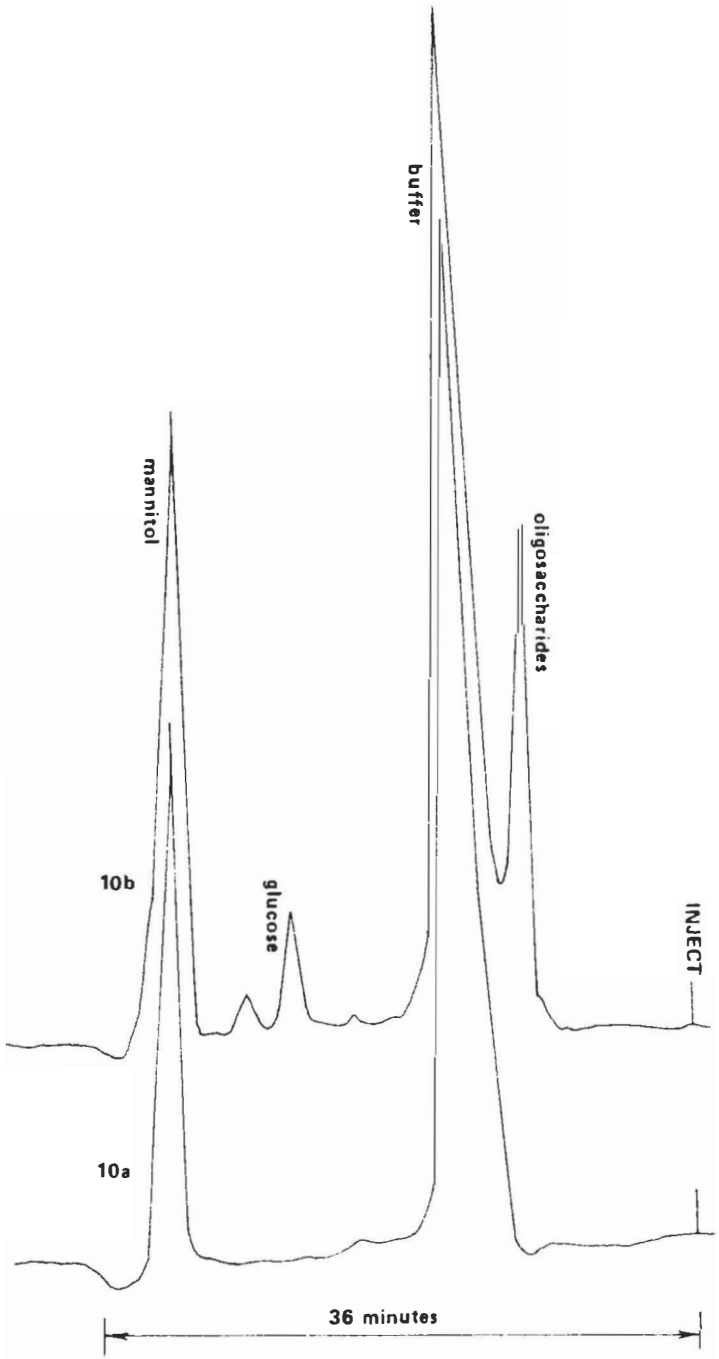


Figure 10. HPLChromatograms of the starch control (10a) and amylase treated starch (10b), both with 30$\mu$g mannitol standard.

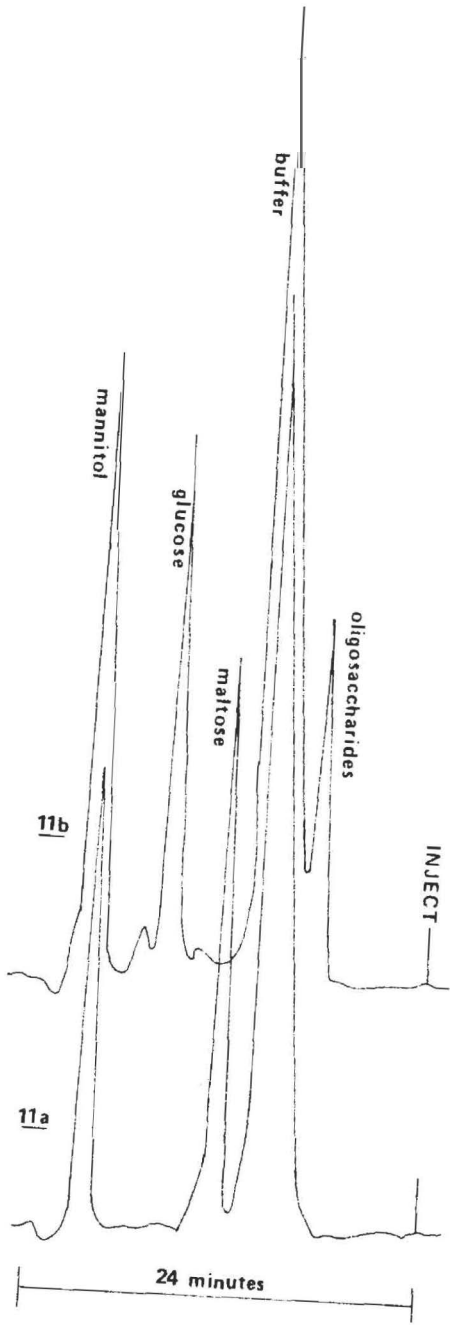


Figure 11. HPLChromatograms of 25μg maltose control (11a) and amylase-treated maltose (11b), both with 30μg mannitol standard.

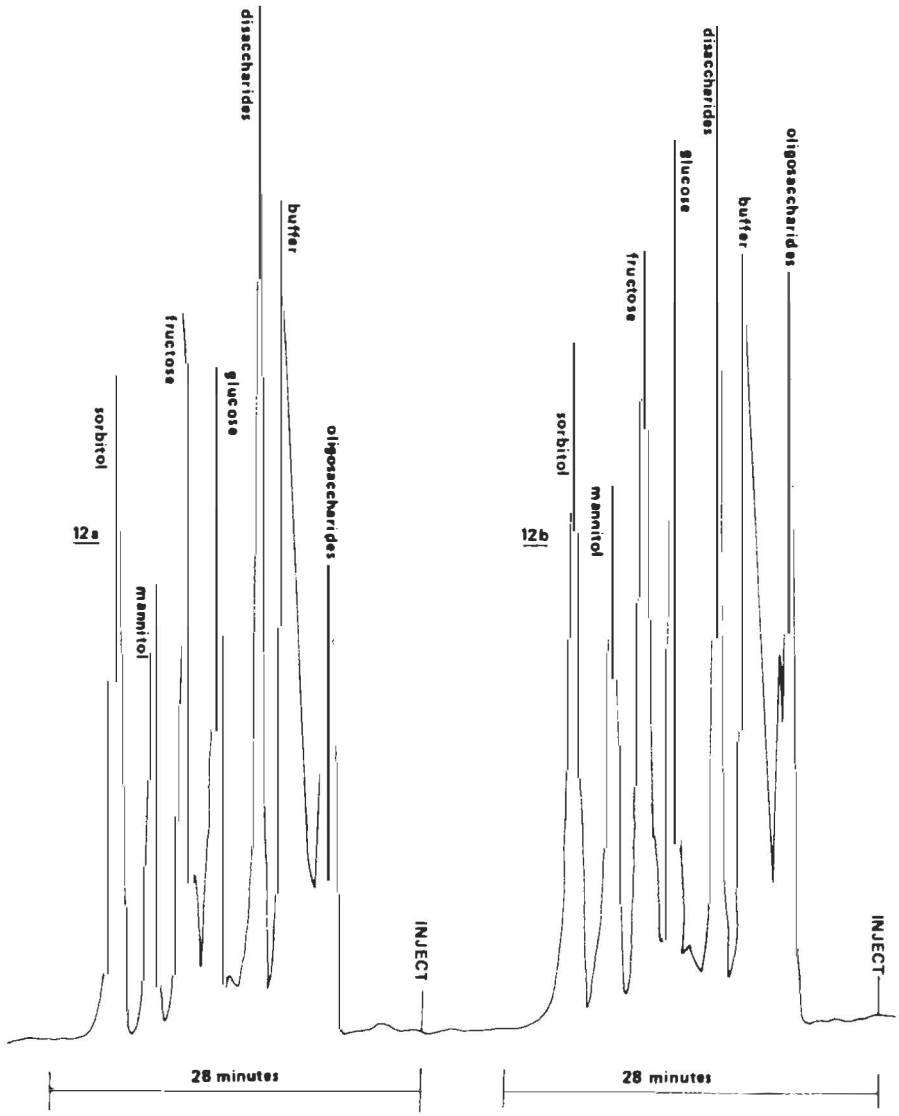


Figure 12. HPLC elution profiles of 10$\mu$l coconut water concentrate (control, 12a) and amylase-treated concentrate (12b), with 30$\mu$g mannitol as internal standard.

in the chromatogram in addition to the internal standard α-methyl-D-glucopyranoside. Based on the retention times of standard sugars the identified peaks correspond to glucose/galactose/mannitol, sorbitol, arabinose, xylose and mannose. However, a few of the observed peaks have not been identified so far.

The gas chromatogram of the alditol acetate derivatives of coconut water (Figure 14b) showed less severe solvent peak tailing and fewer carbohydrate peaks. The identified peaks correspond to glucose/sorbitol, mannose and internal standard which were co-eluted and small amounts of arabinose and galactose.

*Quantitation of Carbohydrate Components*

Although the results of GC analysis could not be quantitated the presence of some sugar components were established. Arabinose, galactose, xylose and mannose were shown to be present using GC, whereas they could not be resolved from one another and from the other sugars by HPLC.

The concentrations of the carbohydrate components of coconut water are given in Table 2, as well as concentration ratios relative to total carbohydrate. For coconut water samples from mature nuts with dried husk the highest concentrations were obtained for sucrose, glucose and fructose. The tabulated values of these concentrations are subject to error due to on-column sucrose hydrolysis as earlier mentioned in this paper. However, the total concentration of sucrose, glucose and fructose in fresh coconut water was found to be approximately constant, i.e. 30 ± 3g/1. Sorbitol is the fourth most abundant carbohydrate component in coconut water followed by oligosaccharides with degrees of polymerization (DP) greater than 5. The total concentration of galactose, xylose and mannose corresponding to peak 8 for fresh coconut water from mature dry nuts was approximately constant at 2.3 g/l. Likewise, the concentration of mannitol was close to 0.3 g/l.

The carbohydrate concentration values for coconut water from mature green nuts (with medium firm solid endosperm) are also presented in Table 2. The total concentration of sucrose, glucose and fructose was equal to 51.5 g/l. The concentration of sucrose was found to be less than half that of either glucose and fructose, unlike in samples of more mature coconut water. Likewise, for less mature coconut water, the concentrations of oligosaccharides (DP $>$ 5) and component 8, as well as the total carbohydrate concentration, are greater than those of more mature samples. These results are in agreement with previous literature reports and explain why coconut water from immature nuts is substantially sweeter than that from mature nuts (Sison, 1984; Banzon and Velasco, 1982; Anzaldo *et al.*, 1975).

It is evident in Table 2 that a wide variability of concentration values was observed for the carbohydrate components of different coconut water samples. The factors for variability include age and variety of coconuts, as well as statistical differences among samples. As previously mentioned, the separate concentration values for sucrose, glucose and fructose are uncertain due to on-column sucrose hydrolysis. Needless to say, further research

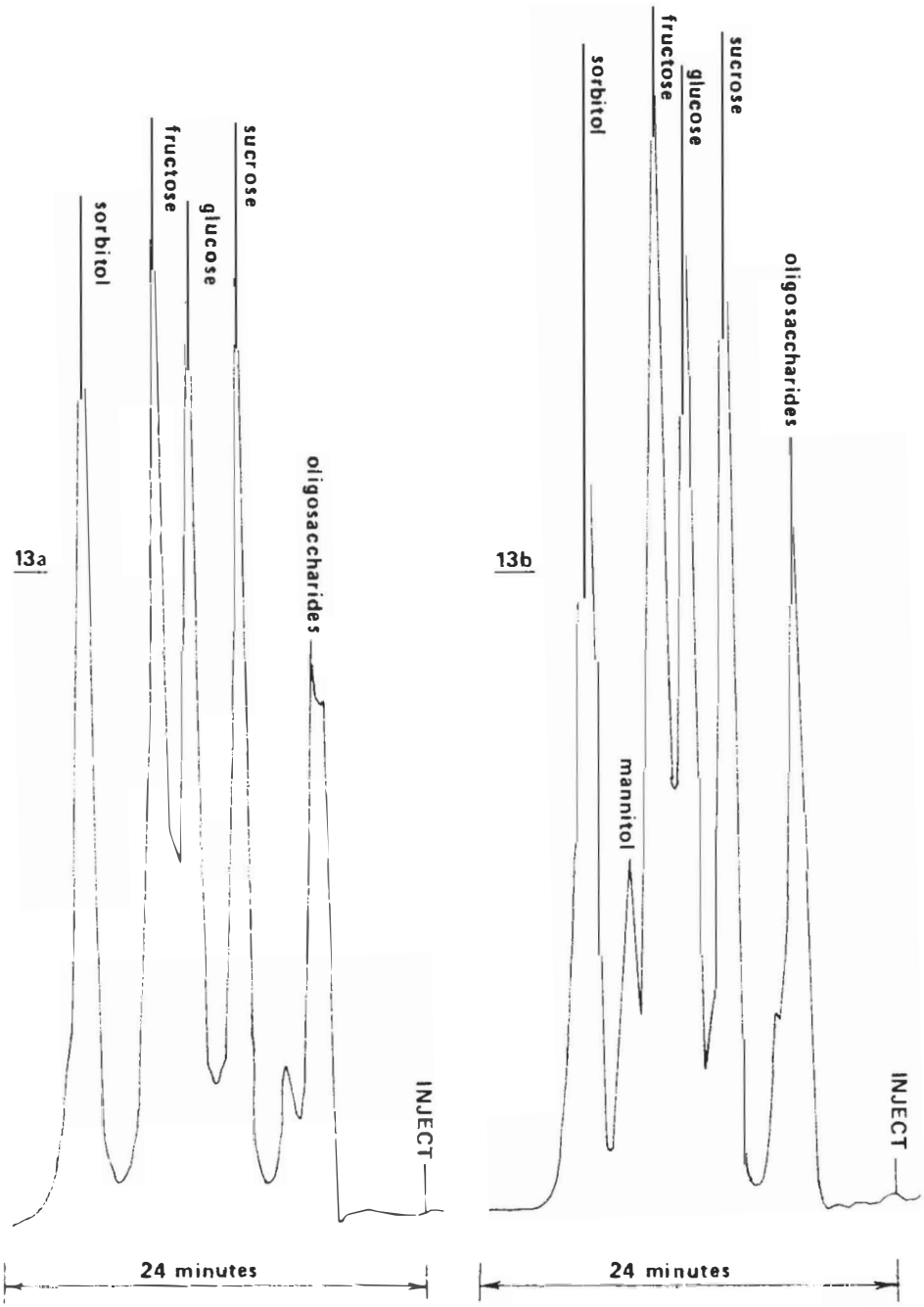


Figure 13. HPLC chromatograms of 3$\mu$l each of coconut water concentrated by reverse osmosis at 30 bars (13a) and 20 bars pressure (13b), with 30$\mu$g mannitol as internal standard.

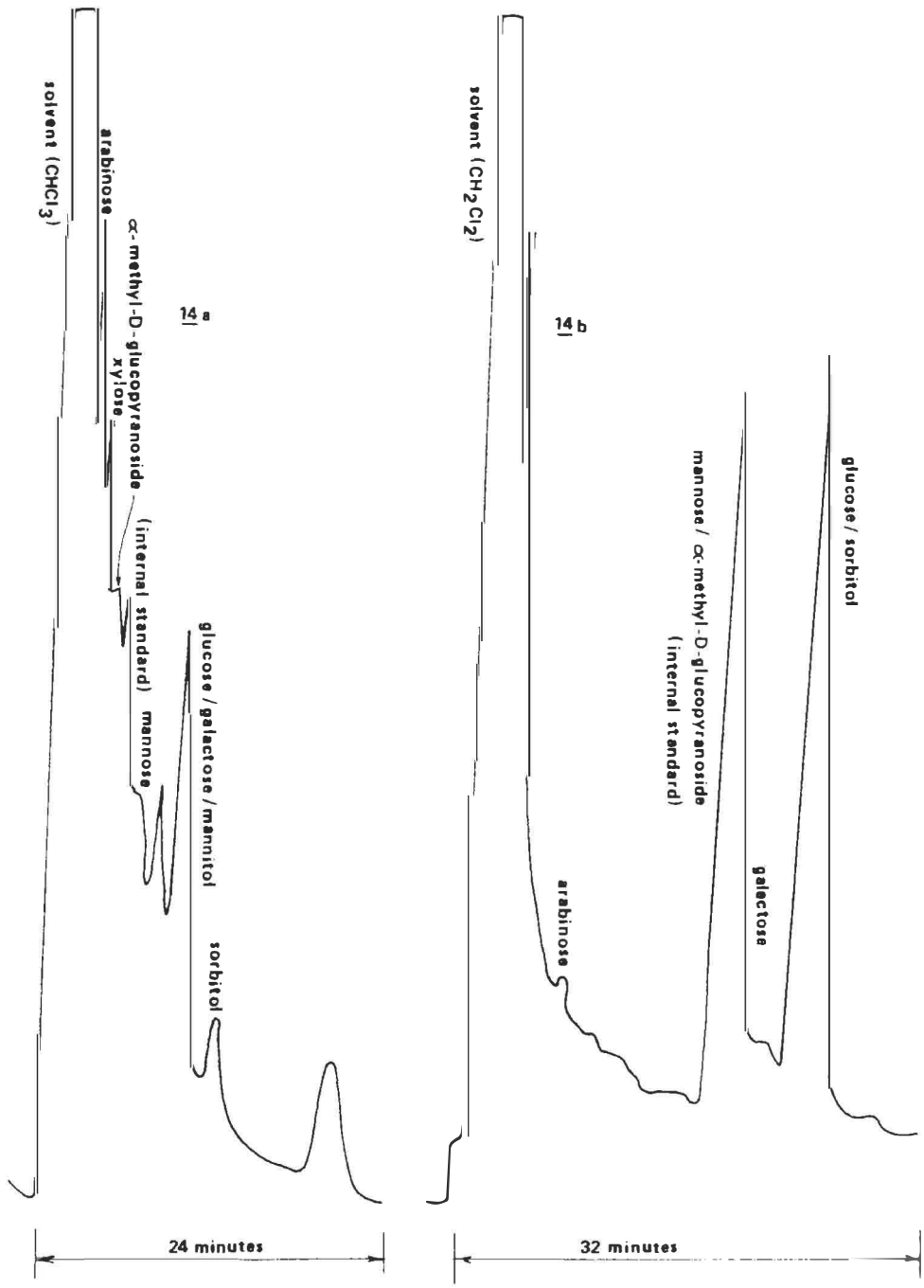


Figure 14. Gas chromatograms of the aldononitrile (14a) and alditol (14b) acetate derivatives of sugars in coconut water concentrate.

Table 2. Actual and relative concentrations of the carbohydrate components of coconut water.*

| Sample | 1 DP>5 | 2 DP>5 | 3 DP5 | 4 DP4 | 5 DP3 | 6** Sucrose | 7 Glucose | 8# | 9 Fructose | 10 Mannitol | 11 Sorbitol | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Coconut water concentrate | 18.80 | 6.32 | 3.67 | 0.59 | 0.59 | 31.40 | 30.55 | 6.17 | 29.52 | 1.41 | 28.94 | 157.96 |
| 30 bars (3 hrs.) | (11.90) | (4.00) | (2.32) | (0.37) | (0.37) | (19.88) | (19.34) | (3.91) | (18.69) | (0.89) | (18.32) | |
| 20 bars (5 hrs.) | 21.21 | 5.57 | 0.85 | – | – | 32.36 | 31.23 | 11.89 | 32.83 | – | 32.17 | 168.01 |
| | (12.62) | (3.32) | (0.51) | – | – | (19.20) | (18.59) | (7.08) | (19.54) | – | (19.15) | |
| Fresh XM300## coconut water | 5.86 | 4.48 | – | 0.18 | 0.15 | 10.82 | 11.30 | 2.23 | 9.45 | 0.27 | 8.28 | 53.02 |
| | (11.05) | (8.45) | | (0.34) | (0.28) | (20.41) | (21.31) | (4.20) | (17.82) | (0.51) | (15.62) | |
| (mature PM30 | 5.29 | 4.05 | – | 0.24 | 0.16 | 9.55 | 9.96 | 2.03 | 8.28 | 0.28 | 7.23 | 47.07 |
| dry nuts) | (11.24) | (8.60) | | (0.51) | (0.34) | (20.29) | (21.16) | (4.31) | (17.59) | (0.59) | (15.36) | |
| C1 | 9.34 | 4.69 | – | 0.38 | 0.27 | 16.78 | 5.72 | 2.72 | 6.10 | 0.35 | 14.98 | 61.33 |
| | (15.23) | (7.65) | | (0.62) | (0.44) | (27.36) | (9.32) | (4.44) | (9.95) | (0.57) | (24.43) | |
| C2 | 7.40 | 5.66 | – | 0.44 | 0.26 | 20.04 | 6.90 | 2.35 | 6.13 | – | 11.57 | 60.75 |
| | (12.18) | (9.32) | | (0.72) | (0.42) | (32.98) | (11.36) | (3.87) | (10.09) | – | (19.05) | |
| Fresh coconut water (mature green nuts) | 5.92 | 7.85 | – | 0.29 | 0.20 | 8.89 | 23.60 | 4.52 | 19.00 | 0.32 | 6.85 | 77.44 |
| | (7.64) | (10.14) | | (0.37) | (0.26) | (11.48) | (30.48) | (5.84) | (24.54) | (0.41) | (8.84) | |

* Actual concentrations expressed in grams/liter; relative concentrations based on total carbohydrate are given in parentheses.

** Calculations were based on an external sucrose standard and not the internal mannitol, due to on-column sucrose hydrolysis (Figure 2a-2b).

\# Galactose, xylose and mannose were co-eluted in peak 8.

\#\# XM30 and PM30 are samples of fresh coconut water which had been passed through these Amicon membranes; while C1 and C2 are two other samples which had been filtered through a Milipore 0.45 μm pore size membrane.

is needed in order to overcome these problems as well as to identify definitively all the carbohydrate components of coconut water. Furthermore, the effect of maturity on the carbohydrate profile of coconut water should be quite interesting in elucidating the physiology of coconut ripening.

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### Florinia E. Merca, Discussant

This paper is supportive of any researches on the possible utilization of the carbohydrates from coconut water not only in yeast production but also in the food industry like in the production of industrial gels. I congratulate Dr. del Rosario and his co-workers on this paper.

Gas Chromatography (GC) is a rapid and accurate method for carbohydrate determination and is also widely used. Similarly, high performance liquid chromatography (HPLC) is indeed an excellent method for carbohydrates analysis. This method is also more rapid compared to GC because there is no need for derivatization – a procedure which entails possible loss of sample.

I have only one comment to make regarding the methodology used in the experiment. I noticed that you were not able to separate the monosaccharides clearly for both the GC and HPLC. I would suggest that perhaps another column be used for GC like Gas Chrom Q with 3% ECNSS which I have used before and obtained good separation. In addition, you can also use a Durrum Anion Exchange Resin for HLPC instead of a sugar – PAK 1 column for separating the monosaccharides arabinose, galactose, xylose and mannose. Sharper detection could perhaps be made by reacting the sample after passing thru the column with a Cu dye reagent Na bichinchoninate and determining its absorbance. A borate buffer pH 8.9 could be used as eluant.

### R.R. del Rosario, Discussant

The author should be commended for a very thorough study on the carbohydrates of coconut water. This is by far the most comprehensive work involving the use of state-of-the-art methods for analysis of carbohydrates in coconut water.

The paper is essentially a comprehensive study on the use of gas-liquid chromatography (GLC) and high performance chromatography (HPLC) for carbohydrate analysis. It presents also the use of internal standards as well as enzyme hydrolysis for identification of unknown fractions.

The interpretation of results however is limited by certain problems in the analytic procedure used. In the case of the HPLC the use of a non-aqueous solvent system might help resolve the hydrolysis of sucrose, which interferes in the quantitative HPLC analysis of sucrose, glucose and fructose. For the GLC separation temperature programming would help separate the individual fractions or peaks and help improve quantitation. The efficiency of the derivatization process of the different sugars is not indicated and therefore may not permit direct comparison with the HPLC data.

In some analyses we have performed on coconut water by HPLC we encountered the presence of inositol. In the analyses presented however, I have not seen

any mention of inositol being present. Another question that may be raised is whether the processing (reverse osmosis) used for concentration affects the composition of the coconut water.