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THE PRODUCTION OF HIGH FRUCTOSE SYRUP
FROM CASSAVA

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Noraini M. Khalid

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Pericles Markakis

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THE PRODUCTION OF HIGH FRUCTOSE SYRUP FROM CASSAVA

By

Noraini M. Khalid

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ABSTRACT

THE PRODUCTION OF HIGH FRUCTOSE SYRUP
FROM CASSAVA

By

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The potential of cassava as a source of high fructose syrup was investigated. Peeled cassava tubers were first subjected to proximate analysis. The starch was then extracted from the tubers by grinding, washing and sedimentation. The calcium content of the starch was 2-3 ppm.

The starch was subjected to enzymatic hydrolysis by a thermostable alpha-amylase and amyloglucosidase to form glucose syrup which was purified by filtration, carbon treatment and ion exchange. At each processing step, the moisture and glucose contents were measured. The syrup was then isomerized by an immobilized glucose isomerase and further purified by filtration, ion exchange and evaporation. Moisture, glucose and fructose contents were measured at each stage of the process.

A forced choice paired comparison method was used to determine the relative sweetness of the high fructose cassava syrup (CS), high fructose corn syrup (HFCS) and sucrose. The triangle test was used to differentiate the two high fructose syrups at equivalent sweetness. To test for preferential differences among the syrups and sucrose, the hedonic scale was used.

The cassava glucose syrup contained 97.7 percent glucose and 2.3 percent maltose and higher saccharides. After isomerization, CS contained 36.5 percent fructose, 61.2 percent glucose and 2.3 percent maltose and higher saccharides. A 13 percent solid solution of CS was found equal in sweetness to 12.8 percent HFCS and 11.4 percent sucrose solution. In the triangle test, CS could be differentiated from HFCS due to the presence of a slight caramel flavor and brown color in CS. There were no significant differences in preference for CS, HFCS and sucrose by the hedonic scale ($p < 0.05$).

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INTRODUCTION

The phenomenal increase in the price of sugar in 1974 (Slater, 1975) has been especially painful for industrial sugar users. The high cost of fertilizers for sugar cane and sugar beet cultivation coupled with the increased demand for sugar were reasons for the increase in sugar prices. The high cost has led many researchers to look for alternative natural sweeteners to relieve the demand for sugar.

In the United States, high fructose corn syrup enjoys this status since it is as sweet as sucrose and cost less. In Japan, due to the shortage of sucrose, a considerable amount of glucose is produced from sweet potato starch and used as a sugar substitute (Takasaki et. al., 1969). Corn is imported into the United Kingdom for the manufacture of high fructose corn syrup since this is more economical than importing sugar (Barker, 1975). In Malaysia, there is sufficient refined sugar to meet the local demand, but sugar cane cultivation is well below the production capacity of the sugar refineries so about 90 percent of the raw sugar used in Malaysia's refineries is imported (Malaysian Business, 1978). Since Malaysia relies heavily on raw sugar imports while there is an abundance of a carbohydrate source in the form of cassava starch, attempts should be directed towards the production of high fructose syrup from cassava.

Cassava production in Malaysia has increased from 218,000 metric tons in 1961-65 to 345,000 metric tons in 1977 (FAO, 1977). The

increase in production has necessitated searching for new outlets to utilize cassava. Converting cassava into high fructose syrup appeared to offer the greatest potential while at the same time decreasing the nation's dependence on imported raw sugar.

Glucose syrups have been used as sweeteners but contain only 74 percent of the sweetening power of sucrose and are therefore not an ideal substitute for sugar. To obtain the same sweetening effect, almost one-and-a-half times the amount of glucose would have to be used with the resultant increase in cost. With the advent of the enzymatic methods of isomerizing glucose to fructose, there is now an economical process for hydrolyzing starch slurry to D-glucose and isomerizing it to D-fructose to form high fructose syrup.

The present study was undertaken to:

(1) Produce a high fructose syrup from cassava starch.

(2) Compare the glucose and fructose contents of the syrup with a commercial high fructose corn syrup.

(3) Compare the relative sweetness of high fructose cassava syrup to sucrose and a commercial high fructose corn syrup.

(4) Determine whether the high fructose cassava syrup was preferred over sucrose and the commercial high fructose corn syrup.

LITERATURE REVIEW

Cassava

The cassava plant (Manihot esculenta, Crantz or Manihot utilissima, Pohl) belongs to the family Euphorbiaceae. It is known as cassava, manioc, mandioca, tapioca and yuca depending on its geographical distribution. The term tapioca, however, is generally used to designate certain forms of cassava products. The plant is primarily cultivated for its tuberous roots which are the cheapest source of carbohydrate known, but young shoots and leaves are also used as food.

The roots usually contain a small amount of hydrocyanic acid which disappears when they are processed for extracting the starch. The action of the enzyme linamarase or of organic acids on the cyanogenic glucosides, linamarin and lotaustralin, present in the roots result in the formation of hydrocyanic acid, acetone and glucose (Wood, 1965; 1966; Clapp et. al., 1966; Bissett et. al., 1969). The glucosides are highly soluble in water and decompose when heated to 150^{o}C while the enzyme is destroyed if the temperature exceeds 75^{o}C. Once hydrocyanic acid is liberated, the roots become detoxicated.

The hydrocyanic acid content varies greatly from 30 to 370 mg/kg roots (Greenstreet and Lambourne, 1933); 29 to 213 mg/kg (Oyenuga and Amazigo, 1957) and six to 190 mg/kg (Wood, 1965). Jones (1959) reported this variation to be due to differences in the growing

conditions, soil, moisture, temperature and age of the plants. The cassava tubers on the average contain 60 to 75 percent water and 20 to 30 percent starch but values as low as 12 percent and as high as 33 percent starch have been reported (Radley, 1976).

Production Trend

The world production of cassava has increased steadily from 85.63 million metric tons in 1968 (FAO, 1969) to 110.17 million in 1977 (FAO, 1977) and occupies 9.79 million hectares in 1968 to 12.57 million hectares in 1977. The total production is probably greater than that reported since cassava is still largely grown by subsistence farmers in small, irregular, scattered plots with much intercropping and undershifting cultivation (Coursey and Haynes, 1970).

Barfoed (1976) reported Brazil, India, Indonesia, Malaysia, Philippines, Thailand, East and West Africa and Madagascar as important producing areas, of which about one-quarter of the total world production is supplied by Brazil (FAO, 1977). In Malaysia, cassava production increased from 218,000 metric tons in 1961-65 (FAO, 1976) to 345,000 metric tons in 1977 (FAO, 1977). The increase indicates the growing importance of the crop and the need for new modes of utilization of cassava.

Utilization of Cassava

The tuber is the primary source of food for human consumption although cassava leaf, which contains up to 30 percent protein on a dry matter basis (Oomen, 1964) is also consumed. According to Coursey and Haynes (1970), cassava is considered as a staple food for about 200 million people in the tropics. Several products have been made

from cassava including macaroni which contains 18-20 percent protein (Bains et. al., 1962), bread and bakery products (Jongh, 1961; Kim and Ruiter, 1969), pasta product (Kwee et. al., 1969) and tapioca starch (Holleman, 1956) which has numerous applications in the manufacturing of salad dressing, sauces, gravies, soups, puddings, pie and cake fillings, biscuits, jello, baby foods and candy.

The potential use of cassava as a substrate for the production of single cell protein has also been investigated (Gray and Abou-el-Seoud, 1966; Brook et. al., 1969). Several workers have also reported the value of cassava as a livestock feed for pigs, cattle and poultry (Oyenuga and Opeke, 1957; Modebe, 1963; Vogt, 1966; Maner et. al., 1967, Cardoso et. al., 1968).

The industrial uses of cassava products include paper and textile sizing, gums, adhesives, glues, dextrins, beer, alcohol, glucose and acetone production. Two good reviews on the utilization of cassava have been presented by Ayres (1972) and Phillips (1974).

Glucose Syrup From Cassava

There is very limited work done on the use of cassava for the production of glucose syrup. As early as 1951, liquid glucose from the acid hydrolysis of cassava starch was produced (Samson, 1951). It conformed with the requirements of the United States Pharmacopoeia for liquid glucose and contained 22.57 percent glucose of 37.76 Dextrose Equivalent (D.E.). Direct acid hydrolysis of cassava starch is, however, unsatisfactory for the production of high dextrose equivalent syrups. This is due to the limited dextrose content and sweetness of the syrups since conversions beyond 58 D.E. have distinct bitter flavors which could not be removed by refining techniques.

Acid hydrolysis also called for subsequent pH adjustments which resulted in the presence of more sodium chloride in the end product.

Enzymes later found their way into the Industry. Park and Papini (1970) used two enzymes - a thermostable alpha-amylase and amyloglucosidase which was low in transglucosidase to convert cassava starch to glucose syrup. The resulting syrup was appreciably sweeter with 98 D.E. and did not have bitter conversion flavors. Enzymatic hydrolysis of cassava starch is also preferred to acid hydrolysis since the former results in higher yields of glucose. It is essential to use an amyloglucosidase which is low or free of transglucosidase since the latter reduces the yield of glucose from starch by forming oligosaccharides with α-(1-6)-glycosidic linkages. The oligosaccharides formed are rehydrolyzed to glucose at a very slow rate. The higher the transglucosidase activity in an amyloglucosidase product, the poorer the conversion of starch to glucose.

The higher yields of glucose obtained from dual-enzyme conversions of starch are in accord with the findings of Lages and Tannenbaum (1978). They reported 100 percent and 99 percent yields of glucose from the enzymatic hydrolysis of cassava starch and cassava meal, respectively.

High Fructose Syrup

Different Carbohydrate Sources for High Fructose Syrups

A review of the developments in the production of high fructose syrups showed that cornstarch has been the pioneer carbohydrate source utilized. High fructose syrup first became available on a limited commercial basis in the United States in 1968 (Francis, 1976).

Initially, a low fructose corn syrup of 82 percent solids containing 14 percent fructose, 43 percent glucose, 31 percent disaccharides and 12 percent higher saccharides was produced with 75 percent the sweetness of sucrose. Within two years, a high fructose corn syrup with a sweetness comparable to sucrose was produced. It had 71 percent solids of which 42 percent was fructose, 50 percent glucose and eight percent higher saccharides. Higher fructose syrups containing 55 to 60 percent and 90 percent fructose are currently available. They are prepared from 42 percent fructose syrups by ion exchange chromatography (Crocco, 1976; Barker, 1976; and Kunin, 1978). This is based on the process developed by Mountfort et. al. (1968) for the separation of fructose and glucose using a sulphonated polystyrene cation exchange resin having a crosslinkage content of four percent divinyl benzene.

High fructose syrup can also be produced from carbohydrate sources other than corn depending on their availability and cost. Vukov et. al. (1977) utilized sorghum and date to produce high fructose syrup. They suggested that date syrup could be used for all foods which required liquid invert sugars provided that their special properties were considered.

Jerusalem artichokes, chicory and salsify extracts have been used successfully for the production of 90 percent fructose syrup (Anon, 1978) but they showed seasonal variability in their fructose-to-glucose ratios. The problem was overcome by diluting the product with glucose to a standard 75 percent fructose and 25 percent glucose syrup. Byun and Nahm (1978), however, obtained a yield of 77 percent fructose and 23 percent glucose from the hydrolysis of Jerusalem artichoke by inulase.

Bananas at different stages of ripeness have been used for the production of high fructose syrup (van Wyk et. al., 1978). When the method similar to that of cornstarch was used, ripe peeled and unpeeled bananas yielded a syrup containing fructose, glucose and sucrose. The presence of sucrose was due to the transformation of banana starch to sucrose upon ripening while the presence of fructose and glucose were attributed to the action of invertase. They observed that over ripe bananas only required a treatment with pectinase followed by separation of insolubles and evaporation for the preparation of high fructose syrup.

Developments in the Production of High Fructose Syrup

Since glucose, the sweetest product derived from starch hydrolysis, is only 74 percent as sweet as sucrose, there was considerable interest in developing corn syrups with high fructose content to increase their sweetness. Efforts were directed at developing enzymes which could isomerize glucose to fructose to produce a high fructose syrup.

Several microorganisms produce such an enzyme. Marshall and Kooi (1957) first isolated a microbial enzyme from cells of Pseudomonas hydrophila grown on xylose. Several problems, however, limited its commercial development such as the requirement of costly xylose for enzyme induction since this enzyme also converted D-xylose to D-xylulose. Toxic constituents-like arsenate were also required in the fermentation medium.

Takasaki (1966) and Takasaki et. al. (1969) overcame the problem of using expensive D-xylose for enzyme production by using

Streptomyces albus. This microorganism has the ability to assimilate xylan, which is less expensive than D-xylose, and produce glucose isomerase. This enzyme is an intracellular enzyme and is therefore costly to obtain because of the processing required to liberate it.

Bengston and Lamm (1972) and Dworschack and Lamm (1972) developed processes for increasing the yields of glucose isomerase from Streptomyces. These have a favorable effect on the economics of the commercial production of high fructose syrup.

Recent developments in enzyme technology have shown that enzymes could be immobilized onto an inert soluble matrix and still retain their activities. Soluble enzyme can be used in only one batch process, but if the enzyme is immobilized, it can be used many times and can be employed in continuous process for the production of high fructose syrup. In addition, immobilized enzymes are generally more stable than their counterparts in solution. The immobilization procedures have been reported by Strandberg and Smiley (1971; 1972); Messing and Filbert (1975); Park and Toma (1975) and Yokote et. al. (1975).

Other microorganisms disclosed in the literature capable of producing glucose isomerase are Lactobacillus brevis (Yamanaka, 1968); Streptomyces olivochromogenes (Kooi and Smith, 1972); Actinoplanes missouriensis (Hupkes and Tilburg, 1976); Streptomyces bikiniensis (Park et. al. 1976); and Streptomyces fradiae, SCF-5 and S. cinnamonensis, MFS-4 (Joseph et. al., 1977).

Application of High Fructose Syrup

High fructose syrup can be used in practically every food which uses liquid sucrose or invert sugar. It is utilized commercially in

still and carbonated beverages, jams, preserves, pickles, salad dressings, ice cream, yoghurt, sauces, pie fillings, soft centred candy, marshmallows, syrups and toppings, canned fruits and baked products.

Levels of sucrose which can be replaced by high fructose corn syrup in various baked products have been indicated by Henry (1976). High fructose corn syrup is an ideal sweetener for the baking industry since yeast-leavened products require high fermentability and residual sweetness. The high monosaccharide contents also contribute to the desirable brown color due to Maillard reaction and caramelization. The ability of the syrup to retain moisture caused by the hygroscopicity of glucose and fructose results in a soft, moist product with extended shelf life.

Sausele et. al. (1976) observed that high fructose corn syrup can totally replace sucrose in bread without affecting the proofing time and loaf volume. Cakes were not texturally altered by 25 percent sucrose replacement by high fructose corn syrup but the crust and crumb colors were darkened due to increased browning reaction.

Volpe and Meres (1976) also reported product browning as a severe problem in using high fructose corn syrup in white layer cakes. This can be overcome by the addition of leavening acids, such as glucono-delta-lactone and sodium aluminimum phosphate-monocalcium phosphate, monohydrate. The resulting cakes were of highly acceptable quality, flavor and with minimum crumb discoloration. Crumb and crust darkening are not as great in dark colored small-sized cakes as in full-size layer cakes due to shorter exposure to baking temperature (Fruin and Scallet, 1975). The decrease in pH from the addition of

leavening acids slowed or inhibited the carbonyl-amino acid reaction due to the loss of the basic amino group.

The cost of sweeteners in carbonated beverages have been reduced by 15 percent when high fructose corn syrup was used (Anon, 1974). The flavor, color and quality of the beverages were unaffected while the shelf life was as good as beverages sweetened with sucrose.

High fructose syrup has a higher osmotic pressure than liquid sucrose due to its high monosaccharide contents (fructose and glucose) and since it is marketed at five to six percent higher total solids than liquid sucrose (Wardrip, 1971). This favors its use in pickle products. The high osmotic pressure will speed equilibrium and minimize cell damage due to the rapid penetration of the sweetener into the cell membranes (Newton and Wardrip, 1974). The high osmotic pressure will also decrease the possibility of bacterial growth once the jar is opened. The color, crispness, flavor and overall appearance of the pickles were excellent.

In the dairy industry, high fructose syrup has been used in ice cream at the rate of 10-30 percent of the total sweetener, while it is used as the primary sweetener in dairy drinks and frozen novelties (Anon, 1975). The problem with the replacement of sucrose with high fructose syrup in ice cream is the depression of the freezing point caused by the lower molecular weight sugar. However, the freezing point is only lowered a fraction of $1^{o}F$ at 50 percent replacement. This effect is usually not detected at the freezer or in the hardening room. To offset the freezing point depression, Robinson (1975) has recommended the use of 36 D.E. corn syrup in conjunction with high fructose corn syrup. The texture of the ice cream produced is

comparable to that made with a standard mix of corn syrup and sucrose. The sweetness levels are balanced to parity with the corn syrup-sucrose mixes while the flavor character is retained.

In yoghurt, less than 100 percent sucrose replacement on an equivalent solids basis has been found to give an equally sweet product (Dingwall and Campbell, 1975). With the fruit flavored variety, there is an enhancement of flavor which is not lost upon storage. Fructose has always been credited with enhancement of natural flavors, especially fruits (Andreas, 1978). The use of high fructose syrup in fruit flavored products will permit the use of less flavor which will result in greater cost savings of sweetener and flavors.

The advent of low calorie and reduced calorie foods will open new possibilities for the higher fructose syrup (90 percent fructose). With the future of the artificial sweetener, saccharin, in doubt, 90 percent higher fructose syrup will be the ideal sweetener for beverages of the "light" nature. The high sweetness level of the syrup, as reported by Andreas (1978) to be 110-150 percent the sweetness of sucrose, enables the use of less sweetener to achieve the desired sweetness, resulting in fewer calories. It is evident that high fructose syrup will be an essential ingredient in food products for many years to come.

Analysis of High Fructose Corn Syrup

Analysis of high fructose corn syrup can be achieved by applying various procedures such as paper chromatography, thin layer chromatography, gas liquid chromatography, high pressure liquid chromatography, nuclear magnetic resonance (NMR) measurements, and specific

enzyme methods. The choice of methodology depends upon the accuracy and sensitivity required. The most useful techniques by far are the chromatographic methods, since they offer the means of studying complex carbohydrate mixtures. Column chromatography is best used for isolating the sugars and paper chromatography for identifying them. Gas chromatography can be used for separating compounds which are volatile or are rendered volatile by derivatization. The method is limited to nonpolar compounds of moderate molecular weight since volatilization occurs without decomposition. Polar compounds such as high fructose syrup with more than one -OH groups tend to decompose or react under gas chromatographic conditions. These compounds have to be derivatized by trimethylsilylation which results in more volatile and more stable polar compounds. However, derivatization is time consuming and where derivatization is necessary, high pressure liquid chromatography (HPLC) will be preferable. HPLC is the most recent technique for separating, identifying and quantifying high fructose syrup since it is sensitive, accurate and fast.

Paper Chromatography

There has been extensive use of paper chromatography especially in the study of reversion products during acid hydrolysis of starch. This technique is the best way of identifying sugar mixtures. Several authors have described quantitative paper cromatography as applied to monosaccharides. Their methods are, however, unsuitable for mixtures of glucose polymers.

A quantitative paper chromatography procedures based on the anthrone reaction for the colorimetric measurement of mono- and

oligosaccharides resolved by paper chromatography and eluted from the paper was described by Dimler et. al. (1952). The results obtained were sufficiently accurate to be within five percent of known sugar mixtures.

Whistler and Hickson (1955) also used paper chromatography for the separation of nine corn syrup components and applied absorptiometric techniques for the quantitative determinations of these components.

The analysis of glucose syrups with paper chromatography is usually preceded by column chromatography. Using this method, eight glucodisaccharides which are considered as reversion products have been separated from a 60 D.E. acid hydrolyzed corn syrup (Ough, 1962). Reversion products are formed by recombination of glucose molecules released during the hydrolysis of starch.

Thin Layer Chromatography

Thin layer chromatography (TLC) was first employed by Stahl and Kaltenbach (1961) for the separation of sugars. Since then, many modifications and improvements in techniques have been published. The major disadvantages of this method include the rather difficult, inaccurate quantitation, the inadequate resolution of a pair of closely running compounds and there is no permanent record of the chromatogram.

The separation of mono- and oligosaccharides on silica gel plates impregnated with sodium acetate, monosodium phosphate and disodium phosphate has been reported (Lato et. al., 1969). Poor separation of oligosaccharides and the low molecular weight trioses and tetroses were obtained while pentoses and hexoses were well separated.

Hansen (1975a; 1975b) described methods for the identification of mono-, di-, tri-, and oligosaccharides in starch hydrolyzates. This is an improvement over Lato et. al.'s (1969) method by the inclusion of lactic acid in the solvent system and using precoated TLC plates impregnated with monosodium phosphate solution. In this system, better separation of oligosaccharides were obtained.

Gas Liquid Chromatography

Many papers regarding the analysis of corn syrups by gas liquid chromatography (GLC) have appeared since McInnes (1958) first reported the separation of carbohydrate derivatives. The trimethylsilyl (TMS) derivatives of sugars developed by Sweeley et. al. (1963) have been widely used. Their method was suitable for the derivatization of mono- and disaccharides while the triose and tetrose components of glucose syrups gave too variable yields for quantitative work.

The development of an improved derivatization method by Brobst and Lott (1966a) permitted the determination of glucose, maltose, maltotriose and maltotetraose in glucose syrups. The separation of TMS derivatives were made with both isothermal and programmed temperature operations, but the latter was unsatisfactory due to unstable baseline encountered during the separation of tri- and tetrasaccharides. Their method should be used with caution since hexamethyldisilazane and trifluoroacetic acid could not be premixed and generated considerable heat and ammonia when reacting.

Brobst and Lott (1966b) improved their existing method by using 60/80 mesh glass beads as support for the liquid phase in a programmed temperature operation. The advantages were lower column temperature,

and no baseline drift. Sennello (1971) used a 21 percent (w/v) solution of N-trimethylsilylimidazole (TSIM) for silylating sugars high fructose corn syrup. The results obtained were unsatisfactory. TMS-fructose appeared as three peaks on OV-17 and as two peaks on OV-101 columns although both glucose and maltose were rapidly and completely silylated. He overcame this problem by using a combination of TSIM and trimethylchlorosilane (TMCS) in pyridine as the silylating reagents. This resulted in fructose being readily silylated and chromatographed as a single derivative and made possible its quantitative determination.

High Pressure Liquid Chromatography

Classical liquid column chromatography was very slow, gave poor resolution due to uncontrolled peak spreading, coning, tailing; and was difficult to quantitate. The problems associated with column chromatography were overcome with the recent developments in microparticulate column packings and equipment for high pressure liquid chromatography (HPLC).

HPLC offers a rapid and accurate analysis of sugars, including oligosaccharides. It equals the precision and accuracy of GLC, but with a minimum of sample preparation. Compared to TLC, HPLC offers much better resolution, sensitivity, accuracy and it produces a chromatographic record which can be readily quantitated. Enzymatic methods are very specific, but the cost of reagents for a large number of analyses is expensive.

Several papers on the use of HPLC for carbohydrate analysis have been published (Brobst et. al., 1973; Lawrence, 1975). Linden and

Lawhead (1975) described the conditions for the resolution and quantitation of glucose, fructose, sucrose, melibiose, raffinose, betaine, three ketose isomers and starch hydrolyzates. Conrad and Palmer (1976) have separated glucose and fructose in high fructose corn syrup using a microBondapak/Carbohydrate packing (10μm pore size) and acetonitrile: water as the mobile phase. Fructose and the shorter chain glucose oligomers in high fructose corn syrup were also separated by elution with water at 70°C from the cation exchange resin Aminex in the Ca^{++} form.

A number of carbohydrates were separated on a Partisil-10 PAC (10um silica gel) packing with acetonitrile: water modified with acid or salt (Rabel et. al., 1976). The modifications in the mobile phase resulted in optimum separation of the saccharides but the theory to explain the effect of the modifications is unclear. The acid or salt added might complex with the carbohydrates or the bonded phase to change the selectivity of the separation.

MATERIALS AND METHODS

Extraction of Cassava Starch

Cassava tubers used in the experiment were purchased from a local store. The tubers were washed, peeled, chopped and ground in a Waring blender and subjected to an extraction procedure modified from Brautlecht (1953), (Figure 1). In the first extraction process, the pulp from the ground tubers was mixed with an equal volume of distilled water and stirred vigorously. Distilled water was used throughout the extraction process. The mixture was passed through a #20 mesh sieve (U.S. Standard Sieve, E.H. Sargent and Co., Chicago, Illinois), shaken and the macerated pulp sprayed with water. Pulp retained on the sieve was reground and again passed through the sieve. The starch milk and fine fibres were collected in a plastic container.

In the second extraction process, the starch milk was screened through a #60 mesh sieve while the meal which remained on the sieve was again ground and screened. Starch milk collected was then passed through a rotating #140 mesh sieve and a brush was used to force the starch milk through the sieve openings. The liquid containing starch was allowed to settle for 30 minutes and the supernatant poured off. The starch was washed three times with five times its volume of water. The pure starch obtained was then dried in a vacuum oven at $60^{o}C$ for 24 hours (vacuum oven Model 29, Precision Scientific

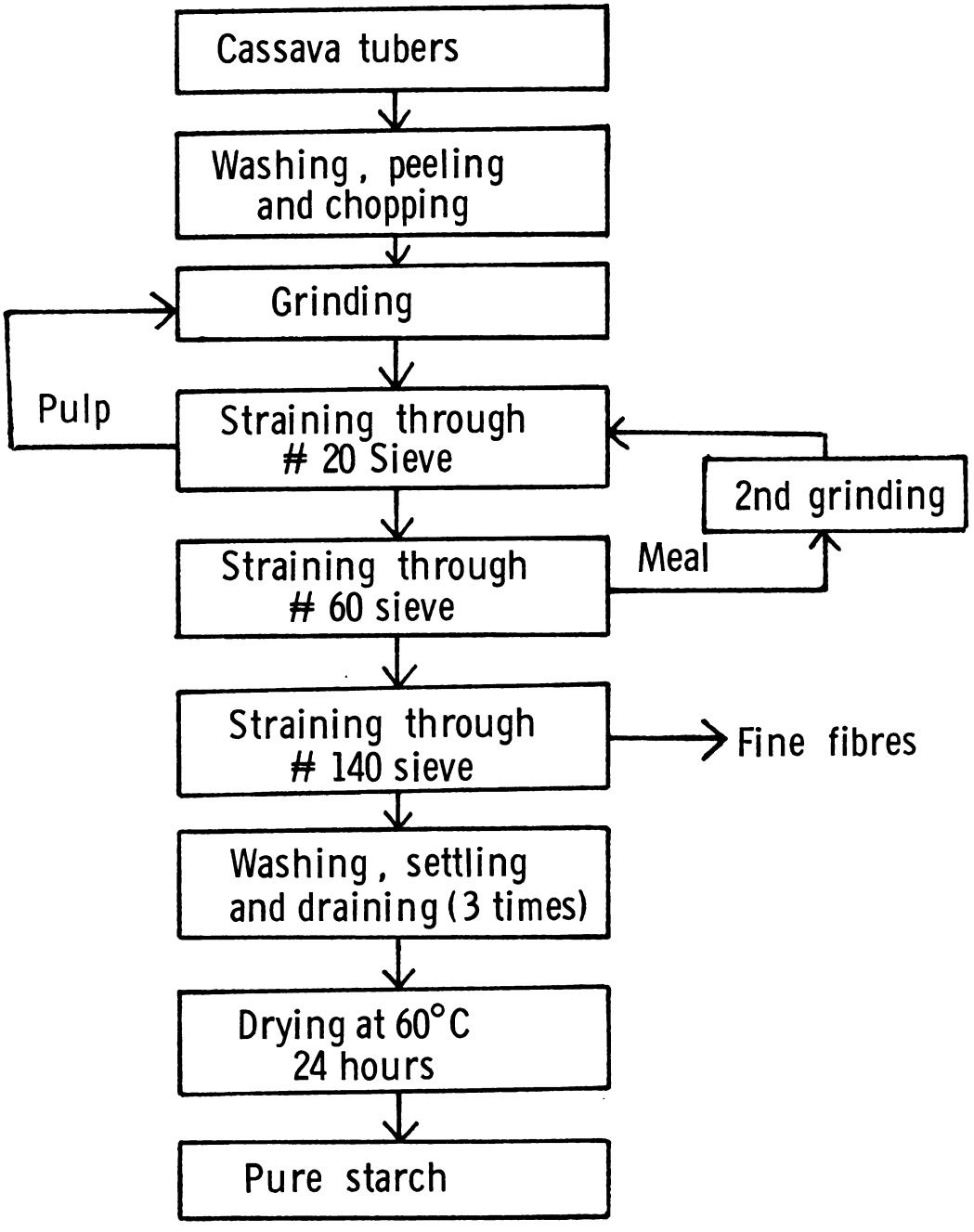

Figure 1 - Flow diagram for the extraction of starch from cassava tubers.

Instruments, Chicago, Illinois equipped with a Cenco Hyvac-2 vacuum pump, Cenco Instruments Corporation, Chicago, Illinois).

Production of High Fructose Cassava Syrup

Materials

Cassava starch was obtained from A.E. Staley Manufacturing Co., Decatur, Illinois.

The following enzymes were supplied by NOVO Laboratories, Inc., Wilton, Connecticut.

(1) Alpha-amylase (Termamyl 60 Liquid)

An exceptionally thermostable alpha-amylase produced from a strain of Bacillus licheniformis. The activity of this enzyme is 60 KNU/g (one Kilo Novo Unit, KNU, is the amount of enzyme which hydrolyzes 5.26 g starch per hour at 37^{o}C and pH 5.7).

(2) Amyloglucosidase (AMG 150 Liquid)

Produced from a strain of Aspergillus niger. It has an activity of 150 AGU/ml (one amyloglucosidase unit, AGU, is the amount of enzyme which splits one micromole of maltose per min. at 25^{o}C).

(3) Glucose isomerase (Sweetzyme)

An immobilized glucose isomerase produced from a strain of Bacillus coagulans. It has an activity of 150 IGIB/g (one unit of immobilized glucose isomerase for batch use, IGIB, is the amount of enzyme which will convert one micromole of glucose to fructose per minute at 65^{o}C and pH 8.5).

Procedure

The process for the production of high fructose cassava syrup is shown in Figures 2 and 3. It is a combination of the methods by Aschengreen (1975), Zittan et. al. (1975) and Lages and Tannenbaum (1978) with several modifications.

Liquefaction

A 33 percent (dry solid basis) starch slurry was prepared by mixing 1980 g cassava starch in 4020 ml of deionized distilled water. The pH was adjusted to 7.0 with 1N NaOH. Alpha-amylase was added to the starch slurry at the rate of 0.125 percent (w/w of dissolved solids) and sufficient calcium chloride was added to give a final calcium ion concentration of 14 ppm. The mixture was stirred continuously with a wooden paddle and heated directly by steam to 100-105^{o}C for 10 minutes to ensure complete gelatinization of all the starch granules. It was cooled to 95^{o}C and with continued agitation using a stirring motor (Precision Scientific Company, Chicago, Illinois), the mixture was held at 95^{o}C for two hours to complete liquefaction by the alpha-amylase. This stage is the dextrinization process.

At this point, a sample of the liquefied starch was drawn off for analysis of glucose and maltose by high pressure liquid chromatography and for moisture content determination.

Saccharification

The liquefied starch was cooled to 25^{o}C and was adjusted to pH 4.9 with 1N HCl. Amyloglucosidase (0.05 percent, vol. of enzyme/wt. of dissolved solids) was added to the substrate and with constant

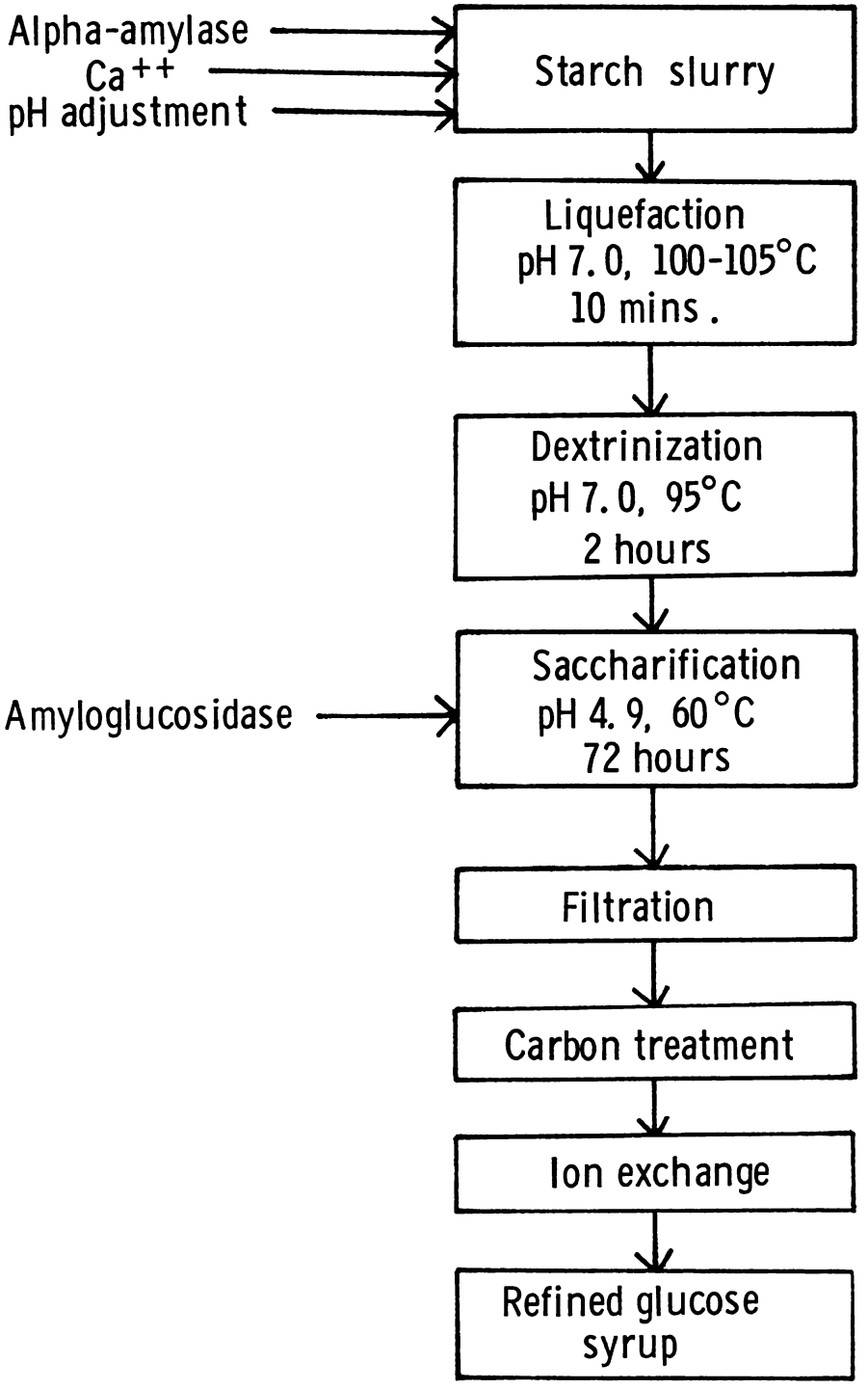

Figure 2 - Flow diagram for the production of glucose syrup from cassava starch.

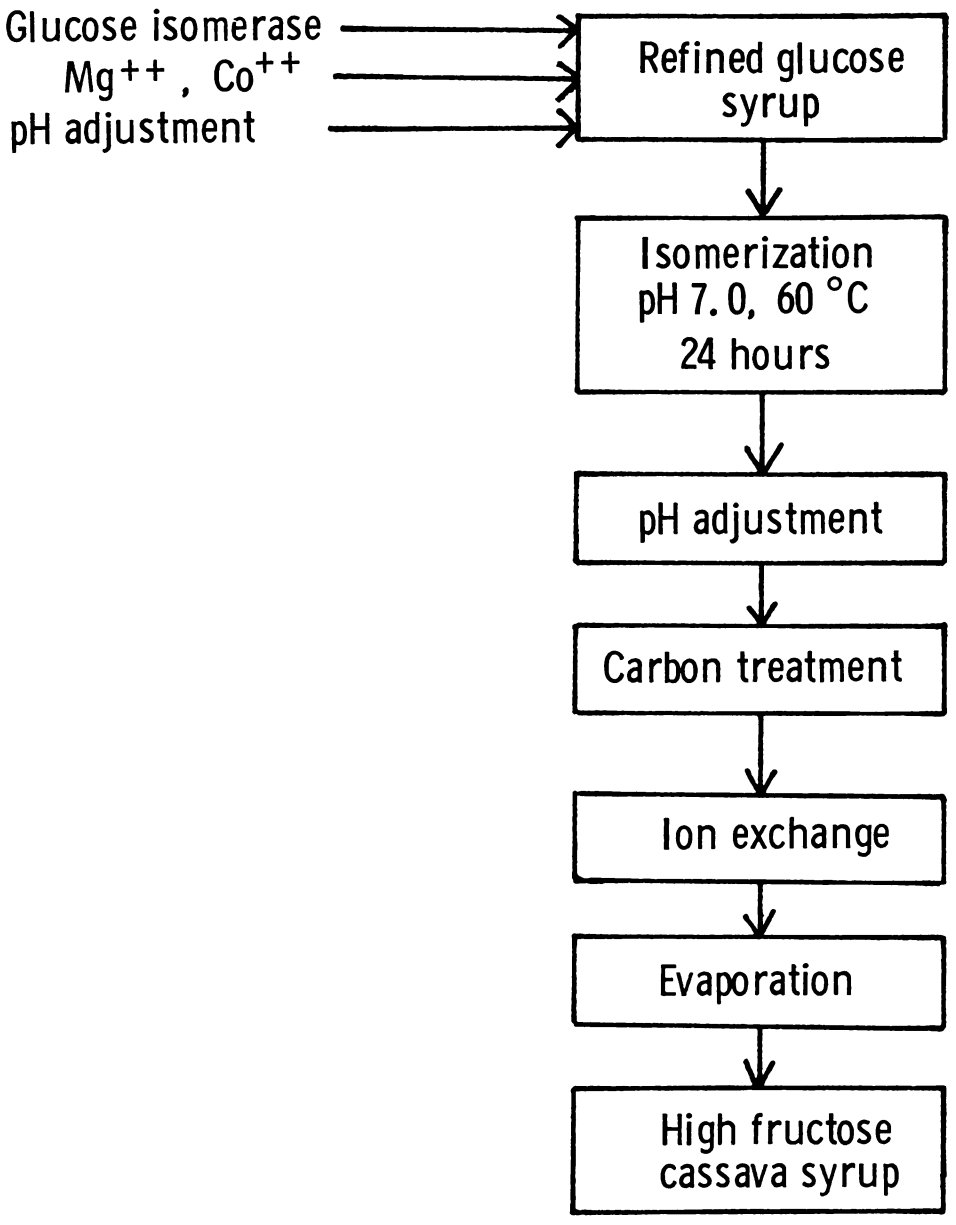

Figure 3 - Flow diagram for the isomerization of glucose syrup to high fructose cassava syrup.

stirring, it was incubated at 60°C for 72 hours in a constant temperature water bath.

Purification

Syrup from the saccharification step was filtered through two layers of Whatman #44 filter paper in a Buchner funnel which was attached to a vacuum pump (Cenco Hyvac-2 vacuum pump, Cenco Instruments Corporation, Chicago, Illinois). The syrup was then filtered through activated charcoal (Matheson Coleman and Bell, E. Rutherford, New Jersey) to remove the color and passed through a cation exchange column (Amberlite CG-50 Type 1, Rohm and Haas Company, Philadelphia, Pennsylvania) which had been washed with 0.5N HCl and with deionized distilled water. This was followed by passage through an anion exchanger (Amberlite IR-45) which had been washed in 0.5N NaOH and in deionized distilled water. The glucose, maltose and moisture contents of the syrup after saccharification, filtration, carbon treatment and ion exchange treatment were determined.

Isomerization

A thorough purification of the syrup was necessary prior to the isomerization process since glucose isomerase is very susceptible to a number of inhibiting compounds including Ca^{++}, Zn^{++}, Cu^{++} (Danno et. al., 1967). The pH of the syrup was adjusted to 7.0 with 1N NaOH. Subsequently, 2 g of $MgSO_4.7H_2O$ and 0.1 g $CoSO_4.7H_2O$ were added in a litre of syrup. If the syrup concentration was not between 40-45 percent solids content, the syrup was evaporated to this solids level in a flash evaporator (Buchler Instruments, Fort Lee, New Jersey) at 38-40°C.

Initially. 0.83 g glucose isomerase was used per kilogram of glucose (Novo Technical Bulletin, 1977). It gave a very low fructose content probably because the enzyme had lost some of its activity. The amount of enzyme used was multiplied by a factor of twenty to 16.6 g enzyme per kilogram glucose. The isomerization was allowed to run for 24 hours at 60^{o}C with constant stirring.

When the reaction was completed, the enzyme was allowed to settle and the syrup was drawn off. A layer of syrup was left to cover the enzyme to prevent its exposure to oxygen. The next batch of syrup was isomerized with the same enzyme.

Purification

The syrup was adjusted to pH 4.5, carbon filtered to remove color, passed through cation and anion exchange columns to remove all the ions and then evaporated to 71 percent solids in a flash evaporator at 38-40^{o}C. The syrup obtained after isomerization and at each step of the purification process was analyzed for glucose and fructose by an enzymatic method and by high pressure liquid chromatography.

Analytical Procedures

Analysis of Peeled Cassava Tubers

The moisture, ash, ether extract and crude protein (N x 6.25) contents of peeled cassava tubers were determined following A.O.A.C. methods (A.O.A.C., 1975). The crude fibre content was determined according to the method described by Pearson (1976). The percentage of total carbohydrate was obtained by subtracting the sum of the percentages of moisture, ash, crude protein, ether extract and crude fibre from 100.

Determination of Calcium in Cassava Starch

The calcium contents of cassava starch prepared in the laboratory and of commercial cassava starch supplied by A.E. Staley Manufacturing Company, Decatur, Illinois, were analyzed using the Beckman DB-G Atomic Absorption Spectrophotometer at 422.7 nm. The method by Perkin Elmer Corporation (1971), for calcium determination in plant products was followed.

Two gram cassava starch was mixed with 3.5 ml perchloric acid, 0.5 ml H_2SO_4 and 15 ml HNO_3. The mixture was digested on a hot plate until fumes of H_2SO_4 came off, indicating that perchloric acid has been eliminated. The residue was redissolved in 2 ml H_2SO_4 and digested again. Lanthanum chloride was added to form a one percent solution. The volume was adjusted to bring calcium to a suitable concentration. The sample was analyzed for calcium. The calibration curve was made from $CaCO_3$ containing 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 10.0 ppm calcium and prepared similar to the samples being analyzed.

Moisture

The moisture contents of glucose and high fructose cassava syrups were determined by the Modified A.O.A.C. method for the determination of moisture in corn syrups (Engel, 1977).

pH

The pH of high fructose cassava syrup was measured with a Corning pH meter, Model 10 (E.H. Sargent and Company, Chicago, Illinois) to $\pm$ 0.01 pH unit.

Ash (sulphated)

Ash content of high fructose cassava syrup was determined by the sulphated ash method (I.C.U.M.S.A., 1964).

Color

The Hunter Lab Color Difference Meter, Model D25-2, was used to determine the color of high fructose cassava syrup and commercial high fructose corn syrup (Isomerose 100 Brand, Clinton Corn Processing Company, Iowa) for the L (lightness), a (redness) and b (yellowness) values. Four measurements were taken on each sample at different points in the syrup.

Determination of Glucose in Cassava Glucose Syrup

Glucose Oxidase Method

The glucose content of cassava glucose syrup at each step of the refining process after saccharification (i.e. filtration, carbon treatment and ion exchange treatment) prior to the isomerization step was determined by the glucose oxidase method (Sigma Technical Bulletin No. 510, 1978). A glucose test kit containing glucose oxidase, horseradish peroxidase, buffer salts and o-dianisidine dihydrochloride, was supplied by Sigma Chemical Co., St. Louis, Missouri. Glucose was obtained from U.S. Biochemicals Corporation, Cleveland, Ohio.

Preparation of Solutions

Enzyme Solution (A)

The contents of one capsule containing 500 International units of glucose oxidase, 100 Purpurogallin units of peroxidase and buffer salts were added to 100 ml distilled water in an amber bottle.

o-dianisidine. 2HCl Solution (B)

Mix 125.0 mg of o-dianisidine. 2HCl in 50.0 ml distilled water.

Enzyme-chromogen Solution (C)

To 1.6 ml of solution B was added 100 ml of solution A.

Standard Glucose Solutions

A series of standard glucose solutions containing 10, 20, 30, 40, 50 and 60 µg glucose/0.4 ml solution were prepared.

Sample Solution

One gram syrup was made up to 100 ml in a volumetric flask with distilled water. The solution was diluted further and 0.4 ml of the final dilution was used for glucose determination. The sample should contain 10 to 60 µg glucose/0.4 ml solution.

Procedure

Pipette Into Tubes	Blank (ml)	Standard (ml)	Sample (ml)
Water	0.4	-	-
Standard Glucose Solution	-	0.4	-
Sample Solution	-	-	0.4
Enzyme-chromogen Solution	4.0	4.0	4.0

Each tube was mixed thoroughly and was incubated at 37°C for 30 minutes. The absorbance of the standard and sample were then measured with a Beckman DU Model 2400 spectrophotometer (Beckman Instruments, Inc., Fullerton, California) against the blank at 450 nm. A calibration curve was made by plotting absorbance at 450 nm against the concentration of standard glucose solutions. The amount of

glucose in the samples were determined from the standard curve. The results were compared to those obtained by high pressure liquid chromatography.

High Pressure Liquid Chromatographic Method

The glucose content of cassava glucose syrup prior to the isomerization process was determined with a high pressure liquid chromatograph (HPLC) at each step of the process (i.e. after dextrinization, saccharification, filtration, carbon treatment and ion exchange treatment).

Determination of Glucose and Fructose in High Fructose Cassava Syrup

High Pressure Liquid Chromatographic Method

Glucose, fructose and maltose contents of high fructose cassava syrup (CS) after the isomerization step, carbon treatment, ion exchange treatment and evaporation were determined with a high pressure liquid chromatograph (HPLC). The HPLC consisted of a Waters Associates pump, Waters Model U6K injector, Waters Model R401 differential refractometer and a Linear Instruments Corporation Model 281 recorder with a chart speed of 0.25 inch/minute. Detector attenuation was 8 X.

The separation of glucose, fructose and maltose were achieved with two prepacked columns joined together. The first was a Whatman Partisil - 10 PAC PXS 10/25 column, 4.6 mm i.d. x 25 cm (Whatman Inc., Clifton, New Jersey) and the second one was a Waters Associates Carbohydrate Analysis Column, 3.9 mm i.d. x 30 cm (Waters Associates, Inc., Milford, Massachusetts).

The mobile phase was acetonitrile: water, 80:20, v/v (Conrad and Palmer, 1976) at a flow rate of 1.15 ml/min. Nanograde acetonitrile was obtained from Mallinckrodt (St. Louis, Missouri). The eluent was degassed and filtered through a Millipore filter (Millipore Corporation, Bedford, Massachusetts) to remove any suspended particles or dust. The pH was adjusted to 5.0 with concentrated phosphoric acid (Rabel et. al., 1976).

Preparation of Standard Curves

Standard solutions were prepared in deionized distilled water from glucose, 10 percent, w/v; fructose, eight percent, w/v (U.S. Biochemicals Corporation, Cleveland, Ohio) and maltose, 10 percent, w/v (Difco Laboratories, Detroit, Michigan) dried overnight in vacuo at 60°C (Palmer and Brandes, 1974). The standard solutions were filtered through a 0.45 μm pore-diameter membrane filter (Gelman Instrument Company, Ann Arbor, Michigan) with a Swinney syringe and filter adapter (Millipore Corporation). Subsequently, 1.0 to 8.0 μl of the standard solutions were injected into the liquid chromatograph with a 25.0 μl syringe (Precision Sampling Corporation, Baton Rouge, Louisiana). Data for the calibration curves were obtained from duplicate analyses.

Sample Preparation

Five gram syrup was diluted to 50.0 ml with deionized distilled water. Samples were similarly filtered as the standards. Ten to 20 μl samples were injected into the instrument depending upon the range of the standard curves made previously.

After separation, the concentration of glucose, fructose and maltose were calculated with reference to peak area standard curves, prepared by injecting known concentrations of these standard sugars.

The glucose and fructose contents of CS obtained by the HPLC method were compared to the corresponding glucose and fructose determinations by an enzymatic method.

Enzymatic Method

The glucose and fructose contents of high fructose cassava syrup (CS) at each step of the process (after isomerization, carbon treatment, ion exchange treatment and evaporation) were determined by an enzymatic method described by Boehringer Mannheim (1977/78).

All the reagents used were obtained from Boehringer Mannheim. The absorbance was measured with a Beckman DU Model 2400 spectrophotometer at 340 nm.

Preparation of Solutions

Buffer

Triethanolamine hydrochloride weighing 14.0 g and 0.25 g $MgSO_4.7H_2O$ were dissolved in deionized distilled water in a 100 ml volumetric flask. The pH was adjusted to 7.6 with ca. 5 ml 5N NaOH and the mixture was made up to the mark with deionized distilled water. The buffer was stable for four weeks at 4^oC.

Nicotinamide-adenine dinucleotide phosphate (NADP)

Sixty mg NADP-Na_2H were dissolved in 6 ml deionized distilled water. The solution was stable for at least four weeks at 4^oC.

Adonesine-5'-triphosphate (ATP)

Three hundred mg ATP-Na_2H_2 and 300 mg $NaHCO_3$ were dissolved in 6 ml deionized distilled water. The solution was stable for at least four weeks at 4^oC.

Hexokinase/Glucose-6-phosphate dehydrogenase (HK/G6P-DH)

Two mg/ml HK were mixed with 1 mg/ml G6P-DH. The undiluted mixture was used. The suspension was stable for at least one year at 4^oC.

Phosphoglucose isomerase (PGI)

The undiluted suspension of PGI (2 mg/ml) was used. It was stable for at least one year at 4^oC.

Preparation of Sample

One gram of syrup was transferred into a 100 ml volumetric flask and made up to volume with deionized, distilled water. The solution was further diluted and 0.1 ml of the final dilution was used for the assay. The solution should contain 3-100 µg glucose + fructose per cuvette.

Procedure

Pipette Into Cuvettes	Blank (ml)	Sample (ml)
Buffer	1.00	1.00
NADP	0.10	0.10
ATP	0.10	0.10
Sample Solution	-	0.10
Distilled Water	2.00	1.90

The absorbance (E_1) was read three minutes after mixing. Then, 0.02 ml HK/G6P-DH was added to the sample and blank, mixed and the time of reaction was noted as zero time. The absorbance (E_2), was read at five minute intervals until the increase in absorbance was constant, indicating that the reaction had stopped. This was followed by the addition of 0.02 ml PGI to both sample and blank and mixed. After 15 minutes, the absorbance of the solutions were read again (E_3).

Calculations

The concentrations of glucose and fructose were calculated according to Appendix A.

Sensory Evaluation

The relative sweetness of high fructose cassava syrup (CS) to high fructose corn syrup (HFCS) and sucrose (Mallinckrodt, Inc., Paris, Kentucky) were determined by a forced choice paired comparison method (Pangborn, 1963). A sample form is shown in Figure 4. This method was also recommended by Stone and Oliver (1969). The judges were requested to determine the sweeter member of the pair, which consisted of a fixed concentration of CS (13 percent solids) and one of the six sucrose solutions at 8, 9, 10, 11, 12 and 13 percent solids. In another test, the 13 percent solution of CS was compared to HFCS (Isomerose 100 Brand, Clinton Corn Processing Company, Clinton, Iowa) diluted to 9, 10, 11, 12, 13 and 14 percent solids.

The panel consisted of 30 members (faculty and students of the Food Science Department). There were 16 males and 14 females, ages ranging from 18 to 55 years. The same panel was used in all the

Date_____

No._____

Judge_____

Relative Sweetness

Instructions: Taste samples in the order presented. Within each pair, circle the number of the sweeter sample. Rinse mouth with distilled water after each pair, NOT within a pair. Do NOT swallow.

Pair Order

1. _____ _____

2. _____ _____

3. _____ _____

4. _____ _____

5. _____ _____

6. _____ _____

THANK YOU

Figure 4 - Scoring form presented to the panel for the forced choice paired comparison method.

tests. All tests were performed in the morning between 10:30 a.m. and 12:00 p.m. in individual partitioned booths equipped with fluorescent and red lights. Red lighting was used to avoid any discriminatory results due to color differences in the samples.

All solutions were prepared in distilled water which had been filtered through two Barnstead Hose-Nipple Cartridge organic remover (# D8904, Barnstead and Company, Boston, Massachusetts) to remove taste and odor. The same water was used for oral rinsing. The solutions were prepared by weight/volume 12 hours before testing and refrigerated before use. No solution was retained for more than 48 hours.

Samples of 15 ml portions were served at room temperature ($21 \pm 2^{o}C$) in odorless plastic cups coded with three-digit random numbers. The serving order within and between the pairs were randomized. The judges were instructed to taste each pair and rinse but not to rinse within a pair.

The equivalent sweetness of CS and sucrose were determined by plotting the percent number of judges selecting various sucrose solutions as sweeter than 13 percent CS, against the sucrose concentration (Pangborn, 1963). The concentration of sucrose at 50 percent of the response (when 50 percent of the judges selected sucrose as sweeter than CS) was designated as the equal sweetness point. The equivalent sweetness of CS and HFCS were similarly determined. The relative sweetness of CS was calculated by arbitrarily assigning a relative sweetness value of 100 to sucrose and HFCS.

Once the equivalent sweetness of 13 percent CS and HFCS had been determined, solutions having equal sweetness were prepared in a lemonade drink mix (Kool Aid lemonade flavor). A triangle test was used to determine whether the judges were able to differentiate between the CS and HFCS of equal sweetness. Three samples were presented simultaneously to each judge with two of the samples being identical and one different. They were instructed to identify the odd sample.

In a third test, CS, sucrose and HFCS were used to prepare solutions of equal sweetness containing also a lemonade drink mix. The hedonic scale method was used to determine whether there was any significant difference among the three samples. The samples were served individually in succession in coded cups. Each sample was tasted and rated on a nine-point hedonic scale (Figure 5) before the next one was served. The hedonic scale was assigned points from one to nine with one representing like extremely and nine dislike extremely. The results were anlayzed by analysis of variance.

HEDONIC SCALE

Date_____

No._____

Judge_____

Instructions: Three samples will be served individually in succession. Each is tested and rated before the next sample is served. The rating you feel the sample should have is to be checked on the nine-point Hedonic Scale.

CODE_____		CODE_____		CODE_____	
Like Extremely	_____	Like Extremely	_____	Like Extremely	_____
Like Very Much	_____	Like Very Much	_____	Like Very Much	_____
Like Moderately	_____	Like Moderately	_____	Like Moderately	_____
Like Slightly	_____	Like Slightly	_____	Like Slightly	_____
Neither Like Nor Dislike	_____	Neither Like Nor Dislike	_____	Neither Like Nor Dislike	_____
Dislike Slightly	_____	Dislike Slightly	_____	Dislike Slightly	_____
Dislike Moderately	_____	Dislike Moderately	_____	Dislike Moderately	_____
Dislike Very Much	_____	Dislike Very Much	_____	Dislike Very Much	_____
Dislike Extremely	_____	Dislike Extremely	_____	Dislike Extremely	_____
Comments		Comments		Comments	

Figure 5 - Scoring Form Presented to the Panel in the Lemonade Drink Comparison

RESULTS AND DISCUSSION

Proximate Analysis of Peeled Cassava Tubers

The nutrient contents of peeled cassava tubers were compared to those found in the literature (Table 1). The values obtained were the means of five replications. On the average, peeled cassava tubers were high in carbohydrate but low in protein, fat, crude fibre and ash. The composition of cassava, as reported in the literature, did not indicate the variety from which the data was obtained. This probably contributed to the variation in the composition of cassava. Variation in the nutrient composition also exist due to differences in the location, environmental conditions and the method of chemical analysis. The variety of cassava used in this experiment was not known since it was obtained from a local store (Figure 6).

Calcium Content of Cassava Starch

No calcium was detected in cassava starch prepared in the laboratory but the commercial cassava starch contained 2-3 ppm of calcium. Raymond et. al. (1941) also did not find any calcium in the starch. It has been suggested by Jones (1959) that the calcium probably was lost during the processing of cassava roots for the extraction of starch.

Figure 6 - Cassava Tubers (scale in inches).

Table 1 - Proximate Analysis of Peeled Cassava Tubers

	SOURCE			
	This Work[a]	INCAP (1968)	CHADHA (1961)	AKINRELE (1962)
Moisture	70.40	66.00	62.50	71.50
Protein (N x 6.25)	0.25	0.23	0.45	0.21
Fat	0.20	0.10	0.10	0.04
Crude Fibre	0.70	0.50	0.50	0.03
Ash	0.95	0.30	0.50	0.20
Carbohydrate (by difference)	27.50	32.90	36.00	28.00

[a]Average of five replications.

Analysis of Cassava Glucose Syrup

Glucose Oxidase Method

The glucose content of cassava glucose syrup, as determined by the glucose oxidase method, is shown in Table 2. It was found that after hydrolysis with alpha-amylase and amyloglucosidase, cassava syrup contained 97.7 percent glucose. The purification steps, which involved filtration to remove any insoluble impurities, carbon treatment to remove color and cation and anion exchange to remove all the ions, yielded syrup containing 97 percent glucose.

The method was based on the catalytic oxidation of glucose by glucose oxidase to form hydrogen peroxide. The latter then reacted with a chromogen, o-dianisidine dihydrochloride, in the presence of

Table 2 - The Glucose, Maltose and Higher Saccharides Content of Cassava Glucose Syrup as Determined by the Glucose Oxidase Method After Four Processing Steps.

Processing Sequence	Absorbance at 450 nm.	Glucose Conc. µg/0.4 ml.[a]	Percent Glucose (dry matter)	Percent Maltose and Higher Saccharide (by difference)
Saccharification	0.3560[b] ± 0.0004[c]	31.75 ± 0.03	97.70 ± 0.10	2.30
Filtration	0.3560 ± 0.0012	31.75 ± 0.10	97.24 ± 0.32	2.76
Carbon Treatment	0.3500 ± 0.0004	31.25 ± 0.03	97.22 ± 0.11	2.78
Ion Exchange	0.3500 ± 0.0004	31.25 ± 0.03	97.15 ± 0.10	2.85

[a]Calculated from linear regression equation: Y = 0.012 X - 0.025 (Figure 7).

[b]Mean of four determinations.

[c]Standard error of the mean.

peroxidase to form a stable colored product which was proportional to the glucose concentration. The reaction is as follows:

$$\text{D-glucose} + O_2 + 2H_2O \xrightarrow[\text{oxidase}]{\text{glucose}} 2H_2O_2 + \text{gluconic acid}$$

$$H_2O_2 + \underset{\text{(colorless)}}{\text{chromogen}} \xrightarrow{\text{peroxidase}} \underset{\text{(brown)}}{\text{oxidized chromogen}}$$

The glucose concentration in the syrup was calculated from a reference curve (Figure 7). To facilitate the calculation, a linear regression equation, $Y = 0.012\ X - 0.025$ was computed, where Y represents the absorbance at 450 nm and X represents the glucose concentration in μg/0.4 ml solution. The glucose values obtained were similar to the values analyzed by high pressure liquid chromatography.

High Pressure Liquid Chromatographic Method

The glucose, maltose and higher saccharides content of cassava glucose syrup after the dextrinization, saccharification, filtration, carbon treatment and ion exchange processes are presented in Table 3. The high pressure liquid chromatographic (HPLC) conditions were similar to those used in the analysis of high fructose cassava syrup.

The hydrolysis of cassava starch by alpha amylase produced a syrup containing 12.6 percent glucose, 32.5 percent maltose and 54.9 percent higher saccharides. The higher saccharides were calculated from the difference of the sum of glucose and maltose from 100. A typical chromatogram for the separation of dextrinized syrup is shown in Figure 8.

After the saccharification process and at each purification step, cassava glucose syrup contained 97.8 to 97.1 percent glucose while

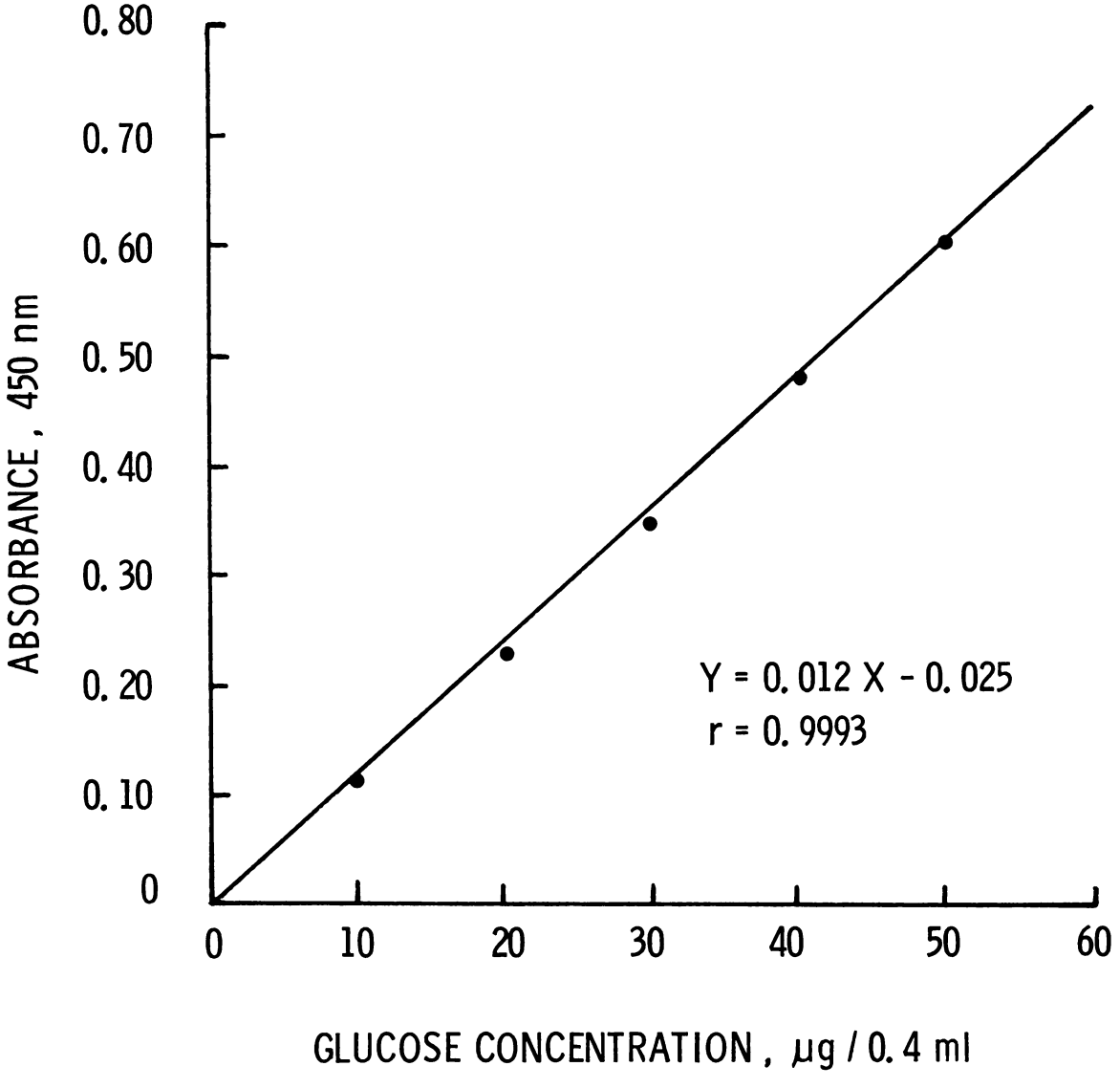

Figure 7 - Reference curve for glucose by the glucose oxidase method.

Table 3 - The Glucose, Maltose and Higher Saccharides Content of Cassava Glucose Syrup as Determined by High Pressure Liquid Chromatography, After Five Processing Steps

Processing Sequence	Moisture[b] (Percent)	Glucose[a]			Maltose[a]			Percent Higher[a] Saccharides (by difference)
		Peak Area (sq. mm)	µg Glucose[c]	Percent Glucose (dry matter)	Peak Area (sq. mm)	µg Maltose[d]	Percent Maltose (dry matter)	
Dextrinization	63.24	495.95	185.18	12.60	845.50	478.40	32.54	54.86
Saccharification	59.38	1076.63	401.78	97.76				2.24
Filtration	59.18	1065.19	379.51	97.38				2.62
Carbon Treatment	59.82	1047.94	391.08	97.30				2.70
Ion Exchange	59.79	1046.50	390.54	97.13				2.87

[a]Average of duplicates.

[b]Average of five determinations

[c]Calculated from linear regression equation: Y = 2.681 X - 0.532 (Figure 12).

[d]Calculated from linear regression equation: Y = 1.8534 X - 41.162 (Figure 14).

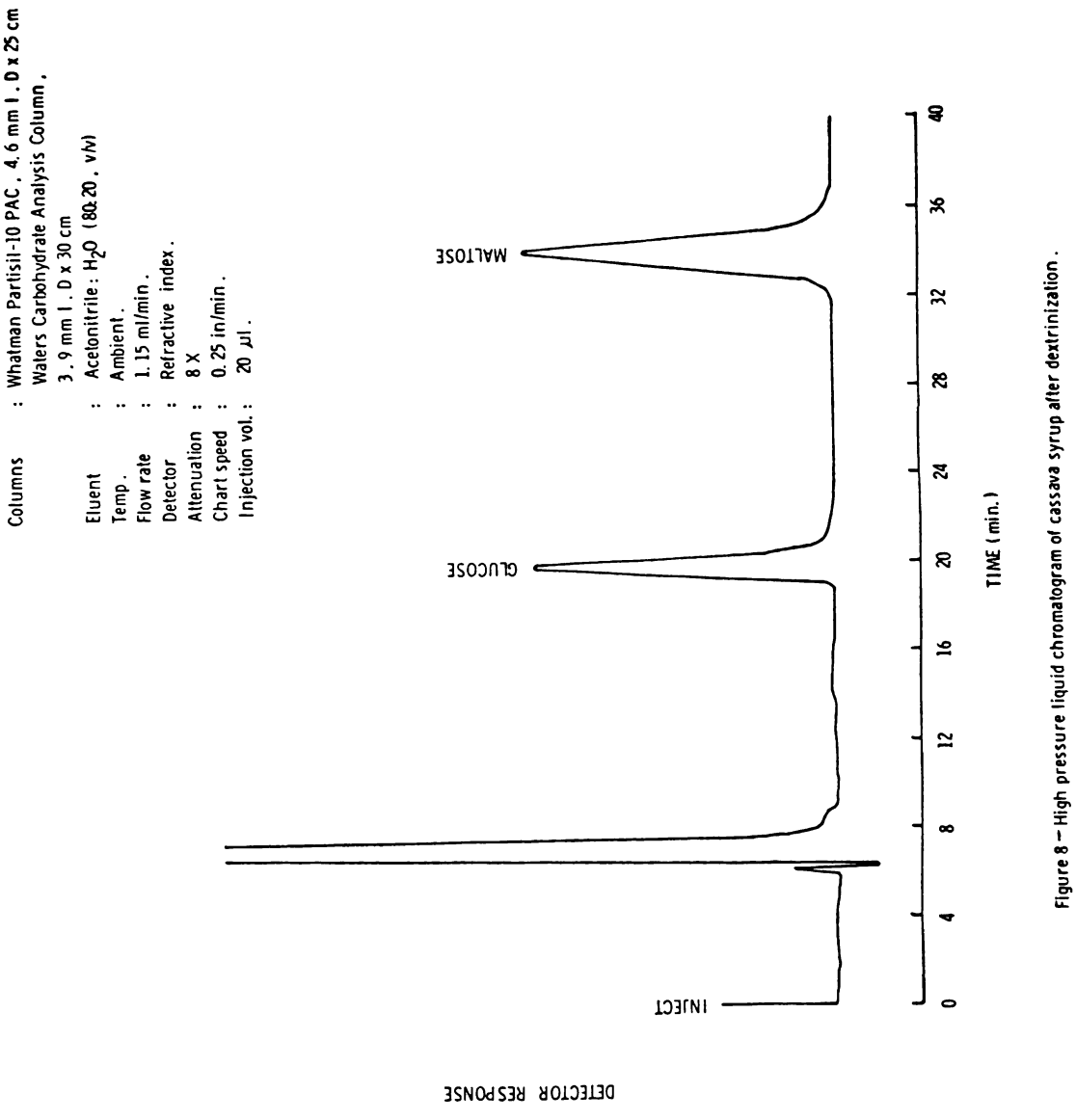

Figure 8 – High pressure liquid chromatogram of cassava syrup after dextrinization.

maltose and higher saccharides were between 2.2 to 2.9 percent (calculated by the difference of glucose from 100). A typical chromatogram for cassava glucose syrup is shown in Figure 9. The results obtained by HPLC were comparable to those obtained by the glucose oxidase method.

Analysis of High Fructose Cassava Syrup

High Pressure Liquid Chromatographic Method (HPLC)

In preliminary trials using one column, the Whatman Partisil-10 PAC, with acetonitrile: water (80/20, v/v) as the eluent, a flow rate of 1.85 ml/min., at ambient temperature, detector attenuation 8 X and the recorder chart speed at 0.25 inch/minute resulted in poor resolution of glucose and fructose in high fructose cassava syrup (CS). Retention times of fructose and glucose were 4.3 minutes and 4.8 minutes, respectively. The times were insufficient to fully resolve the two sugars present in the syrup.

For accurate quantitation of glucose and fructose in CS, there should be an adequate resolution between the two sugars. Very good separation between glucose and fructose was achieved when a second column, the Carbohydrate Analysis Column, by Waters was joined to the Whatman Partisil-10 PAC column. This is demonstrated by the chromatogram in Figure 10. The HPLC conditions remained the same but the flow rate decreased to 1.15 ml/min. Increasing the column length and reducing the flow rate of the eluent resulted in improved separation (Snyder and Kirkland, 1974).

The identities of the sugars in CS were confirmed by injecting pure glucose, fructose and maltose into the HPLC and comparing the

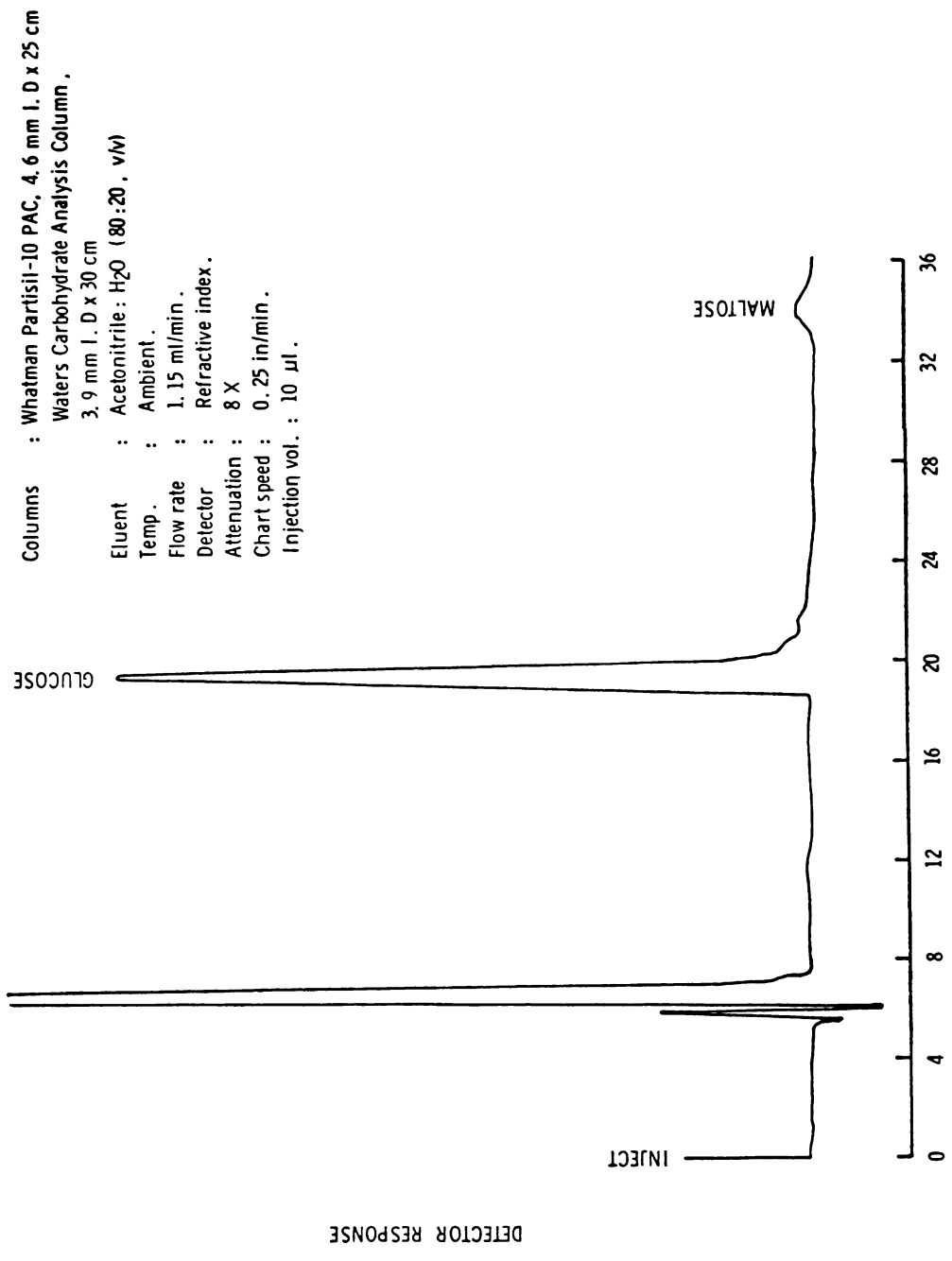

Figure 9 – High pressure liquid chromatogram of cassava glucose syrup.

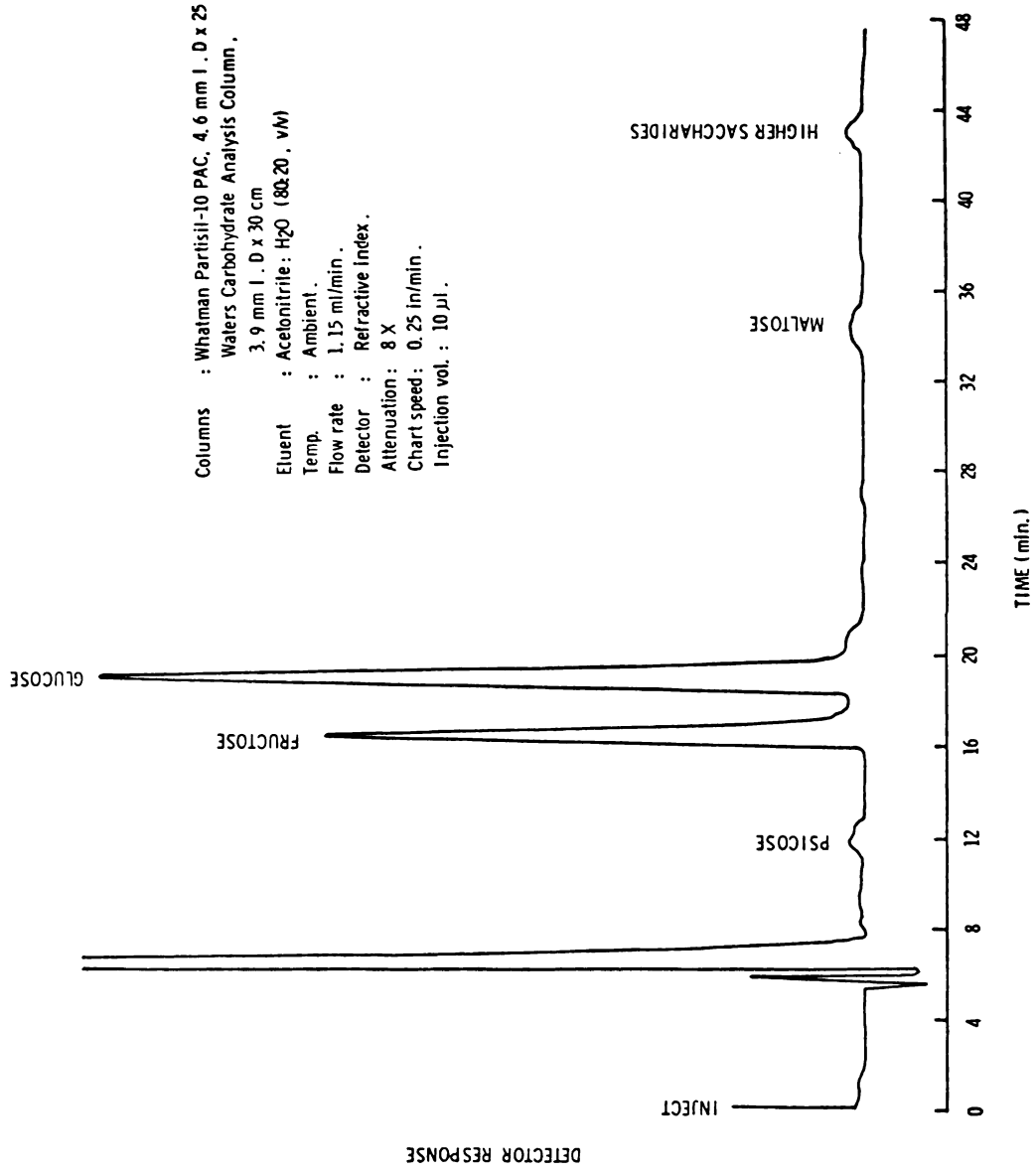

Figure 10 – High pressure liquid chromatogram of high fructose cassava syrup.

retention times of the standards with that of the unknowns. The chromatograms of the standard sugars are shown in Figure 11. The retention times of fructose and glucose were 15.6 minutes and 19.3 minutes respectively, while maltose had a retention time of 34 minutes.

In the chromatogram of high fructose cassava syrup, a small peak appeared after the solvent peak but before fructose was eluted. This could be psicose, a non-fermentable sugar. The presence of psicose in high fructose corn syrup has also been reported by Kooi and Smith (1972) as less than 0.3 percent and also by Conrad and Palmer (1976). No psicose was detected by thin layer chromatography in high fructose corn syrup (Zittan, et. al., 1975).

Standard curves for the sugars were prepared for quantitative analysis by injecting different volumes of known concentration of pure glucose, fructose and maltose in deionized, distilled water into the HPLC. Duplicate analyses were performed. The concentrations of these sugars were directly proportional to the peak area. The reference curves for the three sugars are shown in Figure 12, 13 and 14. Linear regression equations for glucose, fructose and maltose were computed to aid in the quantitation of the sugars, where Y represents the peak area in sq. mm while X represents the microgram glucose, fructose or maltose. The concentration of the sugars in the samples were kept within the range of the reference curves.

Table 4 shows the values for fructose, glucose, maltose and higher saccharides in high fructose cassava syrup. The fructose content after the isomerization process was found to be 36.5 percent while glucose was 61.2 percent. Maltose and higher saccharides accounted for 2.3 percent of the syrup and it was calculated by the

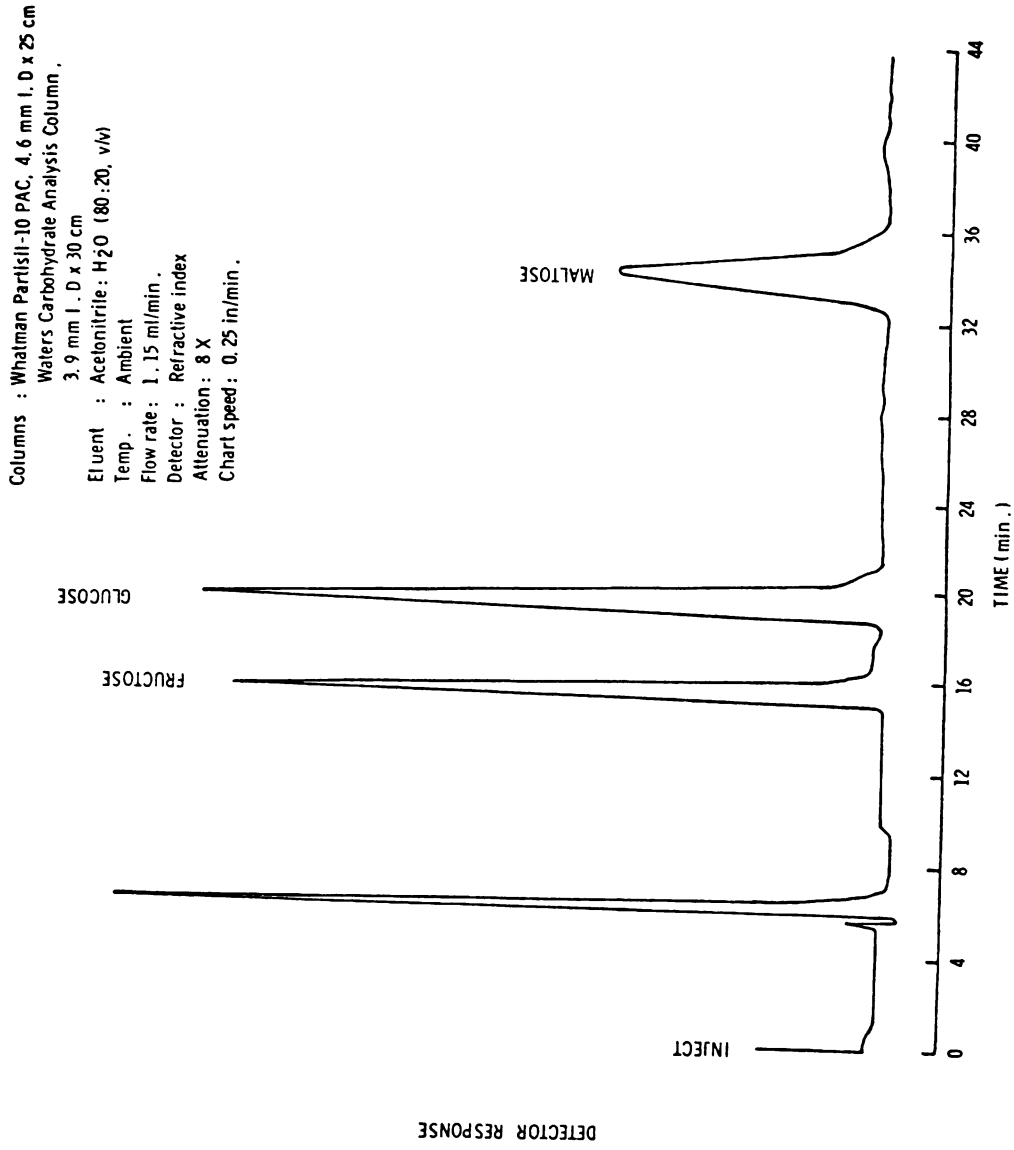

Figure 11– High pressure liquid chromatogram of standard fructose, glucose and maltose.

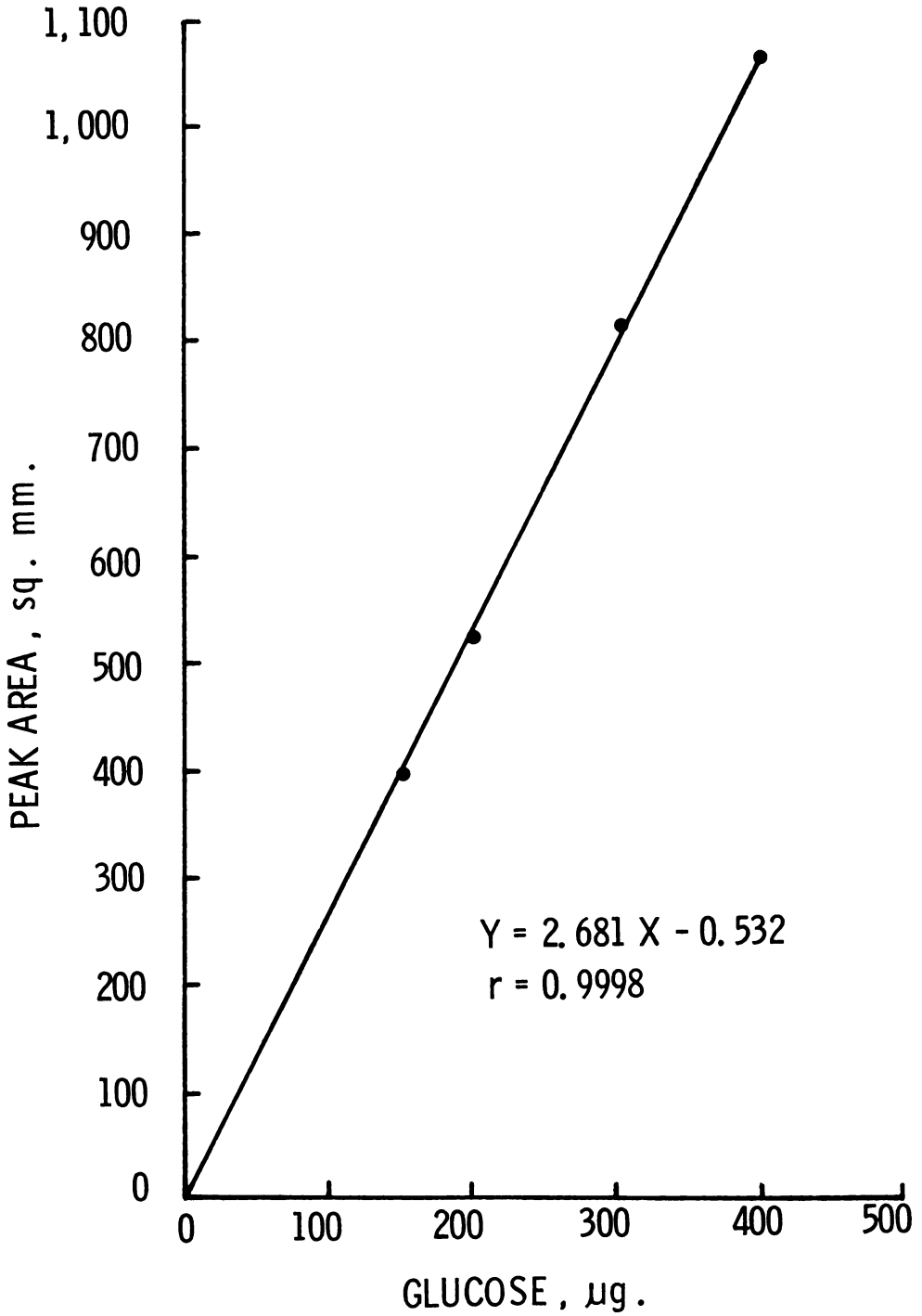

Figure 12 - Reference curve for glucose used in determining this sugar by high pressure liquid chromatography.

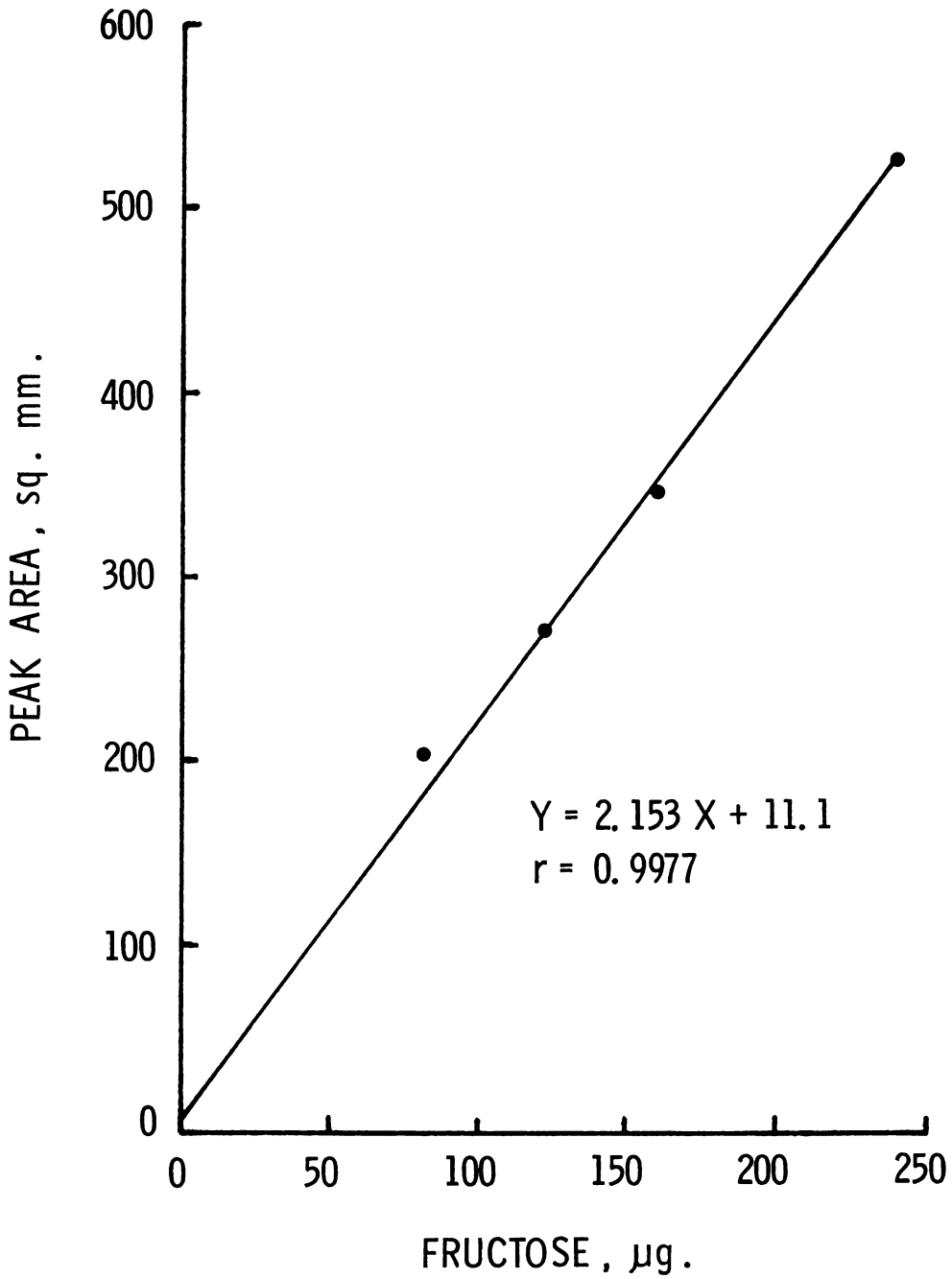

Figure 13– Reference curve for fructose used in determining this sugar by high pressure liquid chromatography.

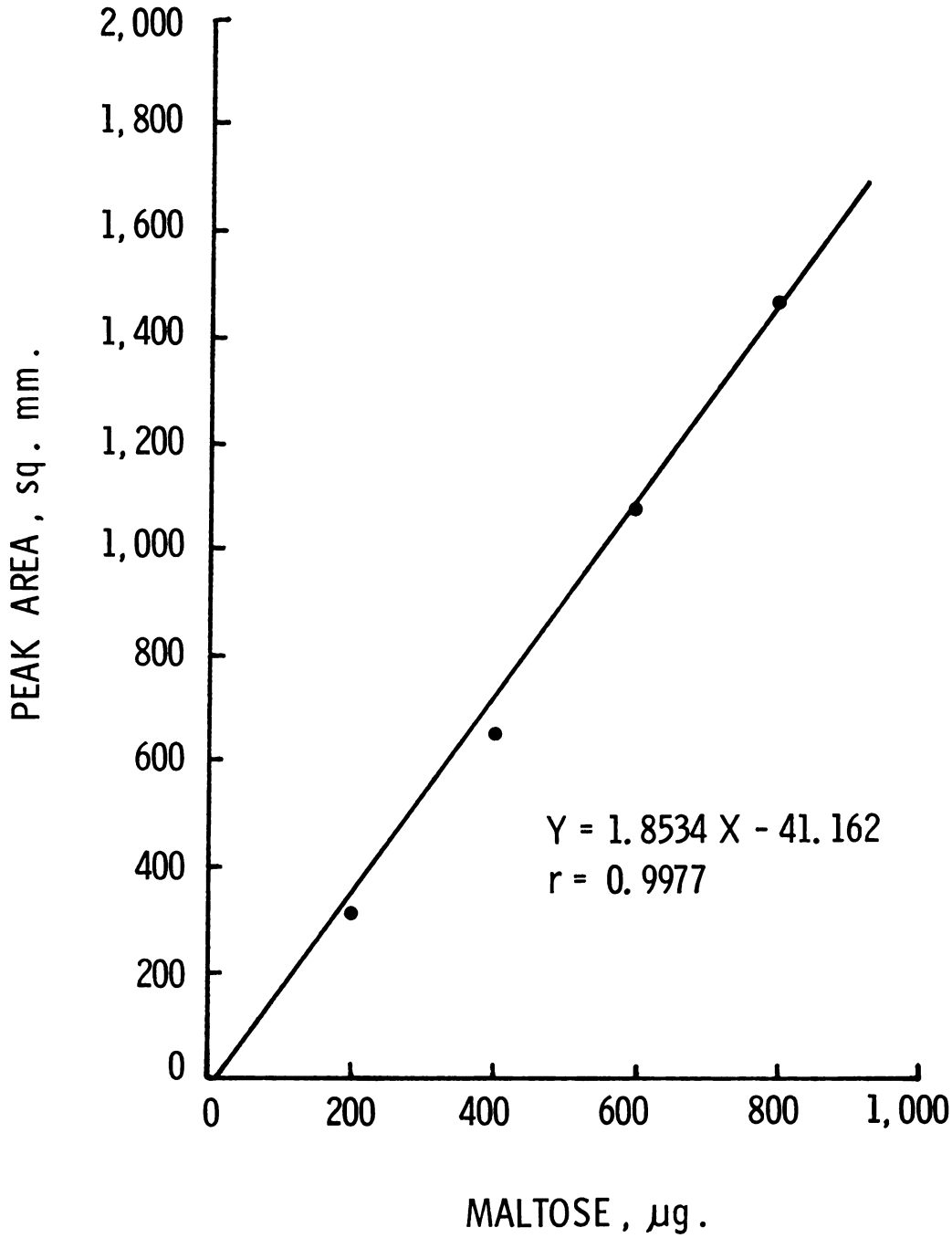

Figure 14 – Reference curve for maltose used in determining this sugar by high pressure liquid chromatography.

Table 4 - The Fructose, Glucose, Maltose and Higher Saccharides Content of High Fructose Cassava Syrup as Determined by High Pressure Liquid Chromatography, After Four Processing Steps.

Processing Sequence	Moisture[b] (Percent)	Fructose[a]			Glucose[a]			Percent Maltose and Higher Saccharides[a] (by difference)
		Peak Area (sq. mm)	µg Fructose[c]	Percent Fructose (dry matter)	Peak Area (sq. mm)	µg Glucose[d]	Percent Glucose (dry matter)	
Isomerization	53.94	378.88	170.82	36.53	766.25	286.01	61.17	2.30
Carbon Treatment	54.00	375.00	169.02	36.64	752.13	280.74	60.85	2.51
Ion Exchange	54.04	377.00	169.95	36.85	749.38	279.71	60.65	2.50
Evaporation	29.30	574.00	261.45	36.90	1155.00	431.01	60.83	2.27

[a]Average of duplicates.

[b]Average of five determinations.

[c]Calculated from linear regression equation: $Y = 2.153 X + 11.1$ (Figure 13).

[d]Calculated from linear regression equation: $Y = 2.681 X - 0.532$ (Figure 12).

difference of the sum of glucose and fructose from 100. The difference method of calculation was used since the peaks for maltose and higher saccharides were too small to quantitate although the presence of maltose in CS has been confirmed by comparing the retention time with that of standard maltose. The syrup was then refined by carbon treatment, ion exchange and finally evaporated to 70.7 percent solids. The glucose, fructose, maltose and higher saccharides contents of the syrup at each step of the purification process were monitored and they agreed with the results obtained after the isomerization process.

Joining the two columns to improve the separation of glucose and fructose resulted in longer retention times of maltose and the higher saccharides. Maltose eluted after 34 minutes while the higher saccharides eluted 42.7 minutes after injection. Increased resolution can also be obtained without any increase in retention time if both the column length and column pressure were increased. This could result in a saving in time per analysis. In this experiment, however, it was not possible to increase the column pressure above 2000 psi as the liquid chromatography unit was not able to operate at much higher pressures.

The peak area method rather than the peak height method was used for the quantitative measurement of the sugars, because peak height is affected more strongly by changes in instrumental parameters such as column pressure, flow rate, etc. (Snyder and Kirkland, 1974).

Enzymatic method

The glucose and fructose contents of CS determined by HPLC were compared to the results obtained by enzymatic analysis (Table 5).

Table 5 - The Glucose[a] and Fructose[a] Content of High Fructose Cassava Syrup as Determined by an Enzymatic Method After Four Processing Steps.

Processing Sequence	Percent Moisture[b]	ABSORBANCE AT 340 nm			Percent Glucose (dry matter)	Percent Fructose (dry matter)
		E_1	E_2	E_3		
Isomerization	53.94	0.090[c] ± 0.000[d]	0.3943 ± 0.0007	0.5750 ± 0.0006	60.84 ± 0.16	36.39 ± 0.06
Carbon Treatment	54.00	0.0825 ± 0.0003	0.3863 ± 0.0005	0.567 ± 0.001	60.80 ± 0.05	36.46 ± 0.11
Ion Exchange	54.04	0.0903 ± 0.0005	0.3933 ± 0.0009	0.5750 ± 0.0004	60.70 ± 0.33	36.64 ± 0.15
Evaporation	29.30	0.0878 ± 0.0005	0.5560 ± 0.0004	0.8373 ± 0.0005	60.93 ± 0.07	36.86 ± 0.03

[a]Calculated according to Appendix A.

[b]Mean of five determinations.

[c]Mean of four determinations.

[d]Standard error of the mean.

The results from both methods agreed with each other. The syrup yielded about 60.8 percent glucose and 36.4 percent fructose after the isomerization process while maltose and higher saccharides was found by difference to be 2.8 percent.

This method involved the phosphorylation of glucose and fructose to glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P), respectively, by hexokinase (HK) and adenosine-5'-triphosphate (ATP).

$$(1)\quad \text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

$$(2)\quad \text{Fructose} + \text{ATP} \xrightarrow{\text{HK}} \text{F-6-P} + \text{ADP}$$

G-6-P was oxidized by NADP to gluconate-6-phosphate with the formation of NADPH in the presence of glucose-6-phosphate dehydrogenase (G6P - DH).

$$(3)\quad \text{G-6-P} + \text{NADP} \xrightarrow{\text{G6P - DH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}^+$$

The amount of NADPH formed in this reaction is stoichiometric with the amount of glucose. NADPH was determined by its absorption at 340 nm. When reaction (3) was completed, F-6-P was converted to G-6-P by phosphoglucose isomerase (PGI).

$$(4)\quad \text{F-6-P} \xrightarrow{\text{PGI}} \text{G-6-P}$$

G-6-P reacted with NADP to form gluconate-6-phosphate and NADPH and the latter was again determined at 340 nm. The amount of NADPH obtained in this reaction was stoichiometric with the amount of fructose.

Sensory Evaluation

In the paired comparison method to determine the relative sweetness of high fructose cassava syrup (CS) to high fructose corn syrup (HFCS) and to sucrose, it was found that CS in water solution of 13 percent solids was equal in sweetness to 11.4 percent sucrose solution (Figure 15) and to 12.8 percent solution of HFCS (Figure 16). This indicated that when sucrose and HFCS were assigned a relative sweetness value of 100 percent, CS was 88 percent as sweet as sucrose while it was 98.5 percent as sweet as HFCS.

The lower relative sweetness of CS as compared to sucrose was due to the presence of glucose, maltose and higher saccharides which are definitely lower in sweetness than sucrose. CS was also slightly less sweet than HFCS because the former has a lower fructose content of 36.5 percent and a higher glucose content of 61.2 percent as compared to the commercial HFCS which has 42 percent fructose and 52 percent glucose.

The concentration of syrup used in the evaluation also affected the relative sweetness. The manufacturers of Isomerose 100 Brand HFCS claimed that their syrup has a relative sweetness of 100 (based on a value of 100 for sucrose) when compared to sucrose in a 15 percent solids solution. This could indicate that if CS with a higher fructose content of 42 percent (as opposed to 36.5 percent) was diluted to 15 percent solids solution, it probably would be as sweet as a 15 percent sucrose solution. Wardrip (1971) also reported that water solutions of HFCS below 15 percent solids were less sweet than sucrose solutions of equal solids content, equal in sweetness at 15 percent solids and sweeter than sucrose above 15 percent solids.

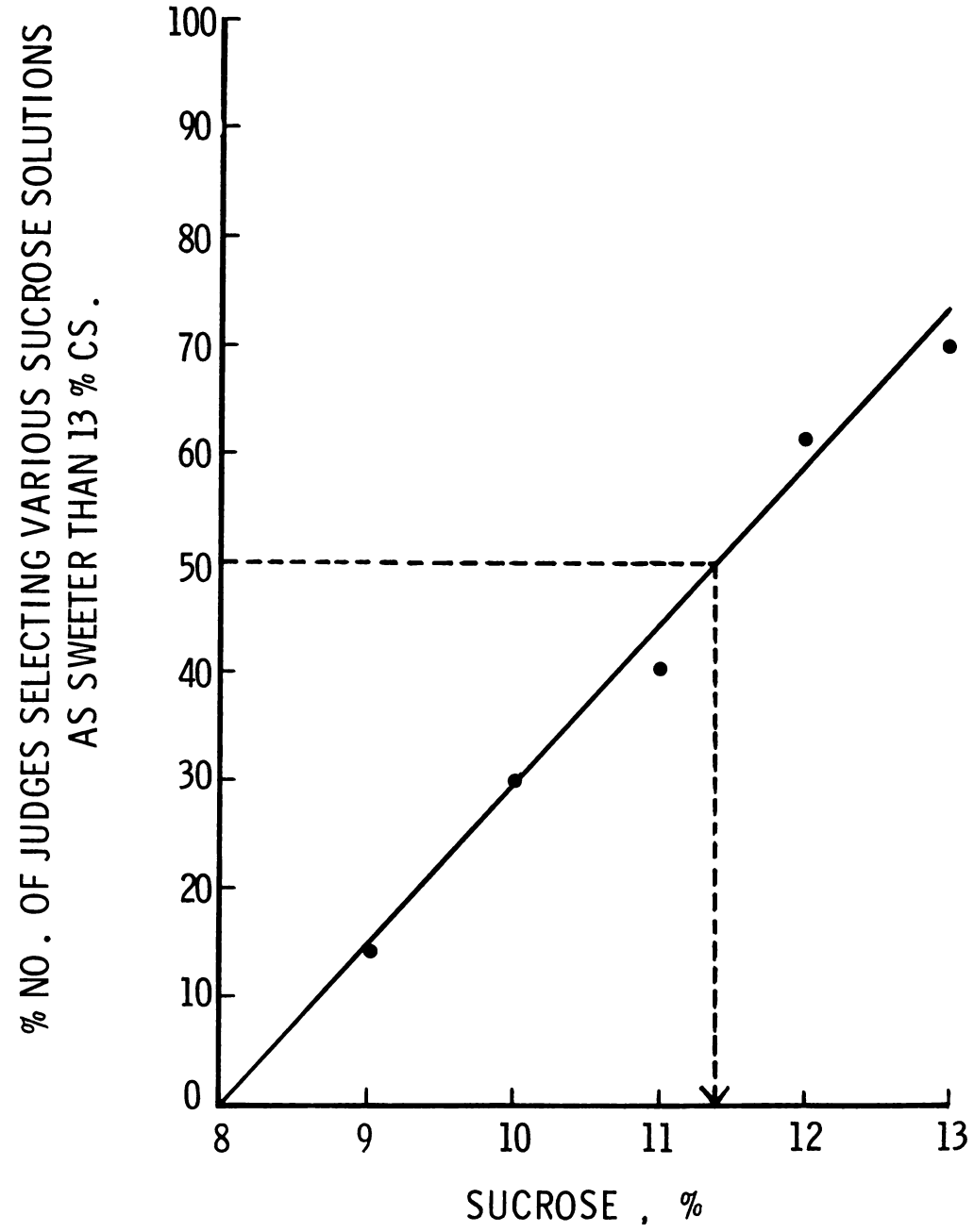

Figure 15 – Graph used in determining the concentration of sucrose having sweetness equal to that of 13 % cassava syrup (CS). No. of Judges : 30.

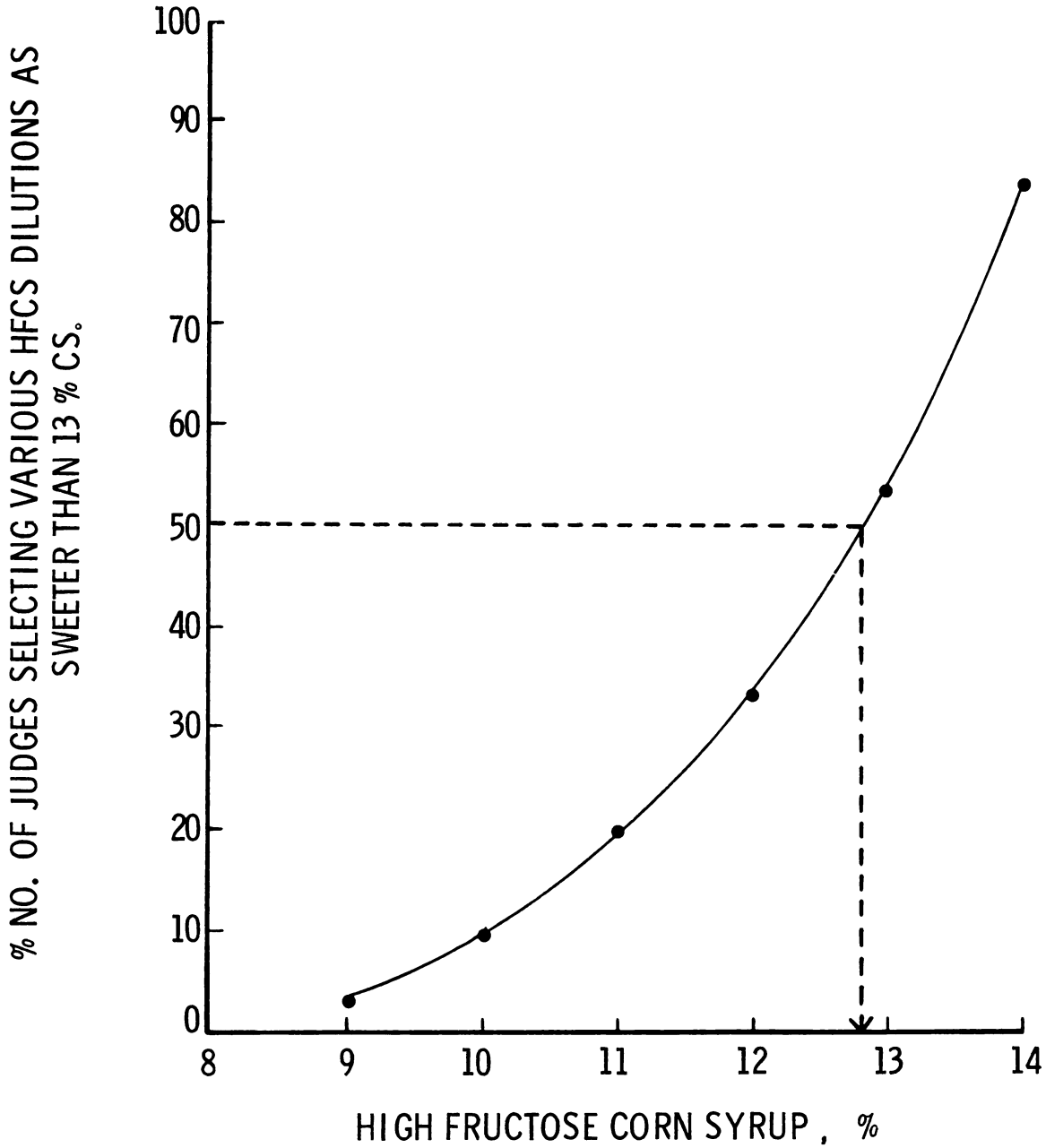

Figure 16- Graph used in determining the concentration of high fructose corn syrup (HFCS) having sweetness equal to that of 13 % cassava syrup (CS). No. of Judges : 30.

In the triangle test, lemonade drinks containing 13 percent CS and 12.8 percent HFCS were prepared. The drinks were equivalent in sweetness. Thirty judges were used in two replications. Out of a total of 60 judgements, 39 of them were correct. From the Table of Significance in triangle tests (Roessler et. al., 1948), since the number of correct judgements exceeded the tabular entry at the five percent level of significance, this indicated that the judges could differentiate CS from HFCS.

The judges made the differentiation based on color and flavor in addition to sweetness. From the comments made, some of the judges were unable to make a distinction between the two samples based on their relative sweetness alone. The presence of a slight caramel flavor and a very light brown color in CS appeared to aid in the differentiation. The color and flavor developed during the flash evaporation of CS at 38-40^{o}C. This could be eliminated by evaporating the syrup at room temperature.

In this test, both syrups were mixed in a lemonade drink since it is known that HFCS has been used commercially to sweeten still and carbonated beverages. Moreover, a more realistic result could be obtained by comparing the sweetness of CS and HFCS in a complex beverage system rather than in water solutions. Pangborn (1963) has also shown that the relative taste intensities of sweeteners was affected by the medium used in dispersing them. She compared the sweetness of sucrose and fructose in pear nectar and found that fructose was less sweet than sucrose at all concentrations, although fructose is known to be sweeter than sucrose.

A third test was run to determine whether there was any significant difference among CS, HFCS and sucrose. A hedonic scale was used. To mask differences in color, yellow food dye was added to the HFCS and sucrose solutions until they approached the color of the CS solution. The test was also done under red lighting. From the analysis of variance (Table 6), there was no significant difference in preference for the three samples, however, among the judges, it was significantly different ($p < 0.05$).

From the comments made, some judges liked CS extremely even though CS had a slight caramel flavor. There was a recommendation that CS be tested in baked products or in confectionery rather than in beverage due to its caramel flavor. Some judges considered sucrose too sour while there were others who thought CS and HFCS as tart.

Table 6 - Analysis of Variance for the Hedonic Scale Test

Source	df	S.S.	M.S.	F
Total	89	143.79		
Sample	2	2.76	1.38	1.35
Judge	29	81.79	2.82	2.76*
Error	58	59.24	1.02	

*Significant at $p < 0.05$.

Considerations for Production Process

The optimum temperature for the activity of alpha-amylase from Bacillus licheniformis is 92°C but in the presence of 30 to 40 percent starch, the heat stability is improved and the enzyme may be used up to 110 to 115°C (Slott et. al., 1974). In this work, the starch

slurry was heated to 100 to 105°C for ten minutes to ensure complete gelatinization as pregelatinized starch is more susceptible to enzyme action. This temperature was sufficient since cassava starch is completely gelatinized at 85°C (Schoch and Elder, 1955).

The enzyme also has a very low requirement for Ca^{++} for maximum heat stability. Slott et. al. (1974) also reported that at 70°C and pH 7.0, less than five ppm of Ca^{++} were sufficient for maximum heat stability. The starch slurry used in this experiment has a final Ca ion concentration of 14 ppm. The small amount of Ca^{++} added would put a smaller load on the ion exchange system since Ca^{++}, being an inhibitor of glucose isomerase, has to be removed.

The purity of the syrup going into the isomerization process is important to the performance of the glucose isomerase. Any impurities might affect the activity of the enzyme adversely. Filtration, carbon and ion exchange refining of the syrup prior to isomerization were thus essential.

The optimum temperature and optimum pH of glucose isomerase from Bacillus coagulans were found to be 75°C and pH 7.0 (Danno et. al., 1967). Isomerization, however, was carried out at 60°C because the reaction at higher temperatures would induce intensive color formation and decomposition of fructose. The same workers also reported that the enzyme activity was not affected below 70°C but was completely inactivated at 90°C.

Zittan et. al. (1975) observed a higher optimum pH range of 8.0 to 8.5 for glucose isomerase activity. In this work, the isomerization was performed at pH 7.0 in order to avoid excessive browning. Below pH 5.0, the enzyme is irreversibly denatured.

Initially, 0.83 g glucose isomerase was used per kilogram of glucose. The isomerization was run for 24 hours but the yield of fructose was too low. The enzyme could have lost some of its activity as it was about a year old. The amount of enzyme used later was multiplied by a factor of 20 and the syrup containing 36.5 percent fructose and 61.2 percent glucose was obtained. The amount of enzyme used might appear to make the process uneconomical. However, Zittan et. al. (1975) have used 23 to 26 g of similar enzyme per kilogram glucose for the first isomerization batch. They obtained a syrup with 45 percent fructose and 55 percent glucose. The immobilized glucose isomerase was reused for subsequent isomerization process and the activity decreased after five to six reuses.

The refining process which included carbon and ion exchange treatments removed all the color and the ions which contributed to the ash content of the syrup. The refined high fructose cassava syrup was bright, clear and colorless. A light brown color developed when the syrup was evaporated to 70.7 percent solids content at 38 to 40^{o}C. The concentrated syrup obtained was not filtered through carbon as it was too viscous. It would be possible to eliminate the color by flash evaporation at room temperature. A slight caramel flavor also developed as a result of the caramelization of the syrup at the temperature of evaporation. A comparative analysis of the high fructose cassava syrup and commercial high fructose corn syrup is shown in Table 7.

Table 7 - Comparative Analysis of High Fructose Cassava Syrup (CS) and Commercial High Fructose Corn Syrup (HFCS)

	% CS	% HFCS [1]
Total Solids	70.7	71.0
Moisture	29.3	29.0
Color [2]		
L	+ 5.8	+ 5.4
a	+ 0.9	+ 1.0
b	+ 0.43	- 0.25
	Dry Basis Composition	
Carbohydrate Components		
Fructose	36.5	42.0
Glucose	61.2	50.0
Higher Saccharides	2.3	8.0
Ash (sulphated)	0.02	0.03
pH	4.6	4.3
Relative Sweetness [3]	98.5	100

[1] Data for Isomerose 100 Brand high fructose corn syrup is from: Schnyder, B.J., 1974. Die Starke. 26:409-412.

[2] Color measured by the Hunter Lab Color Difference Meter, Model D25-2.

[3] Compared to HFCS in a 13 percent solids solution.

Yield

The yield of the conversion of cassava starch to glucose was:

$$\frac{1406 \text{ g glucose}}{1366 \text{ g starch}} \times 100 = 103\%$$

The true yield is: $\frac{180}{162} \times 100 = 111\%$

The yield of glucose to fructose was:

$$\frac{506 \text{ g fructose}}{1406 \text{ g glucose}} \times 100 = 36\%$$

The theoretical yield was:

$$\frac{36.5 \text{ g fructose}}{97.7 \text{ g glucose}} \times 100 = 37.4\%$$

The 36 percent yield of fructose was not very different from the 42 to 45 percent yield obtained in the Industry. The low yield obtained in this work was probably attributed to the process parameters not being optimized yet.

The lower yield of conversion of starch to glucose and also of glucose to fructose obtained in the practical operation was due to losses during the handling and manipulation of the syrup.

SUMMARY

The purpose of this study was to produce a high fructose syrup from cassava starch and to compare it to commercial high fructose corn syrup.

Cassava tubers were peeled and subjected to proximate analysis. The starch was extracted from the tubers by grinding, washing and sedimentation. The calcium content of the starch was determined to be 2-3 ppm.

A 33 percent (dry solid basis) starch slurry containing 0.125 percent (wt./wt. of dissolved solid) alpha-amylase and 14 ppm Ca^{++} was heated to 100 to 105°C for 10 minutes at pH 7.0. It was then cooled and further incubated at 95°C for two hours.

A 0.05 percent (vol. of enzyme/wt. of dissolved solids) amyloglucosidase was added to the liquefied starch which was then incubated at 60°C, pH 4.9 for 72 hours. The glucose syrup obtained was filtered, decolorized and passed through ion exchange columns.

The refined syrup was adjusted to pH 7.0 Bivalent ions in the form of $MgSO_4.7H_2O$ (2 g/L syrup) and $CoSO_4.7H_2O$ (0.1 g/L syrup) and 16.6 g glucose isomerase per kilogram glucose were added. The syrup was isomerized for 24 hours at 60°C. The high fructose syrup obtained was adjusted to pH 4.5, filtered through carbon, passed through ion exchange columns and evaporated to 70.7 percent solids at 38 to 40°C.

The cassava glucose syrup obtained was analyzed for glucose by the glucose oxidase method and by high pressure liquid chromatography. The glucose content of the syrup after each purification step was also monitored. Cassava glucose syrup contained 97.7 percent glucose and 2.3 percent maltose and higher saccharides.

The high fructose cassava syrup (CS) was also analyzed for glucose and fructose by an enzymatic method and by high pressure liquid chromatography. The syrup contained 36.5 percent fructose, 61.2 percent glucose and 2.3 percent maltose and higher saccharides.

The relative sweetness of CS to high fructose corn syrup (HFCS) and sucrose were determined by a forced choice paired comparison method. It was found that CS in water solutions containing 13 percent solids was equal in sweetness to 11.4 percent sucrose solution and 12.8 percent solution of HFCS.

CS and HFCS having equal sweetness were prepared in a lemonade drink. A triangle test was used to differentiate the two high fructose syrups at equivalent sweetness. CS could be differentiated from HFCS due to the presence of a slight caramel flavor and light brown color in CS.

The hedonic scale method was used to determine whether there was any significant difference among the CS, HFCS and sucrose samples. There was no significant difference in preference for the three samples, however, among the judges, it was significantly different ($p. < 0.05$).

The high fructose cassava syrup obtained had a total solids content of 70.7 percent, 36.5 percent fructose, 61.2 percent glucose, 2.3 percent maltose and higher saccharides, 0.02 percent ash and pH 4.6. The color of syrup was measured with a Hunter Lab Color

Difference Meter since it had a slight brown color. The L value was + 5.8, the a value was + 0.9 and the b value was + 0.43.

This work indicates that an acceptable high fructose syrup could be prepared from cassava. The process would certainly upgrade the value of cassava starch, which has been very low up to this time in many cassava producing countries.

APPENDIX

APPENDIX A

Calculation of glucose and fructose values by the enzymatic method:

(1) The differences in absorbance ($E_2 - E_1$) for the blank and sample were determined as follows:

(a) $E_{2S} - E_{1S} = \Delta E_S$ Where: S = sample, B = blank

(b) $E_{2B} - E_{1B} = \Delta E_B$

(2) $\Delta E_{(glucose)} = \Delta E_S - \Delta E_B$

(3) The differences in absorbance ($E_3 - E_2$) for the blank and sample were determined as follows:

(a) $E_{3S} - E_{2S} = \Delta E_S$

(b) $E_{3B} - E_{2B} = \Delta E_B$

(4) $\Delta E_{(fructose)} = \Delta E_S - \Delta E_B$

(5) The concentrations of glucose and fructose were calculated using the formula:

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta E \ (g/l)$$

Where: V = final volume (ml); 3.22 ml for glucose and 3.24 ml for fructose

v = sample volume (0.10 ml)

MW = molecular weight of glucose or fructose (180.16)

$\Delta E = \Delta E_{(glucose)}$ or $\Delta E_{(fructose)}$

d = light path (1 cm)

ε = extinction coefficient of NADPH at 340 nm is 6.3 L $mmol^{-1}$ cm^{-1}

For glucose:

$$c = \frac{3.22 \times 180.16}{6.3 \times 1 \times 0.1 \times 1000} \times \Delta E_{(glucose)} \quad g \text{ glucose/L sample}$$

For fructose:

$$c = \frac{3.24 \times 180.16}{6.3 \times 1 \times 0.1 \times 1000} \times \Delta E_{(fructose)} \quad g \text{ fructose/L sample}$$

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