AN INVESTIGATION INTO THE SUCROSE AND REDUCING SUGAR CONTENT OF DIFFERENT FRUITS

PRESENTED BY

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ABSTRACT

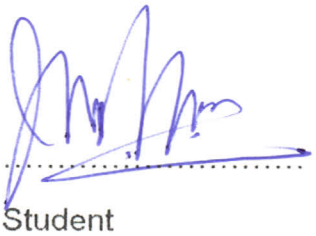
Sucrose and reducing sugar contents were determined in two varieties each of pineapples, mangoes and oranges using Lane-Eynon titrimetric method. Sugars in the fruits were separated by descending paper chromatography and detected by using silver nitrate in acetone.

All the fruits contained sucrose and the order of decreasing sucrose content was mandarin, Keitt variety mango, local seedling mango, sugar loaf pineapple, Smooth Cayenne pineapple and sweet orange. The reducing sugar content was fairly similar in all the fruits.

Paper chromatographic separation indicated the presence of sucrose, fructose glucose, arabinose and mannose. Ribose, galactose and maltose were not detected in any of the fruits.

DECLARATION

The experimental work presented in this project report was performed by me at the Food Research Institute and the Department of Biochemistry, University of Ghana, Legon under the supervision of Dr. Jonathan Adjimani.



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Student

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Supervisor

June, 1998

DEDICATION

This project report is dedicated to the Almighty God JEHOVA, the creator of all things including fruits.

ACKNOWLEDGEMENT

I am much grateful to my supervisor Dr. Jonathan Adjimani and Mr. Clement Christian, both of Biochemistry department, University of Ghana, for their immense contributions that enabled me to complete this project.

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CONTENTS

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Carbohydrates are widely distributed in nature. They are formed during photosynthesis, a process in which solar energy is converted into chemical energy in living matter. Carbohydrates, composed of carbon, hydrogen and oxygen are therefore the primary source of metabolic energy for living organisms. A carbohydrate is an aldehyde (-CHO) if the carbonyl oxygen is associated with a terminal carbon atom, and a ketone (>C = 0) if the carbonyl oxygen is bonded to an internal carbon. Carbohydrates are therefore polyhydroxyaldehyes and polyhydroxyketones.

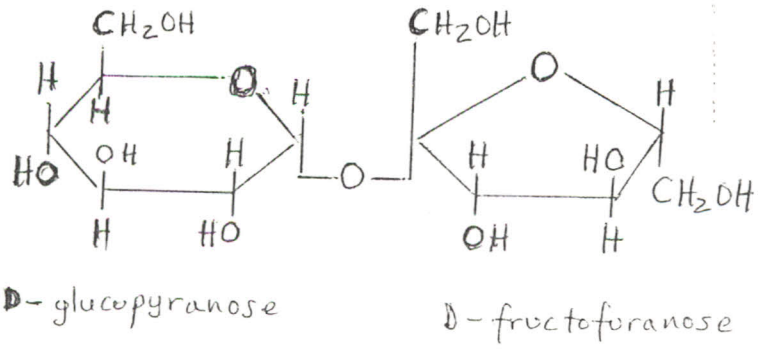
Carbohydrates are classified as monosaccharides, oligosaccharides and polysaccharides as they occur in nature. The word saccharide comes from the Greek word "sakcharon" meaning sugar. Monosaccharides are therefore carbohydrates that contain only one sugar unit and cannot be broken down into other simple sugars. Examples of monosaccharides are glucose and fructose. Oligosaccharides are molecules containing two to ten monosaccharide units, i.e. disaccharide (2 units), trisaccharide (3 units), and so on. They can therefore be broken down into simpler units when hydrolyzed. Polysaccharides are larger polymeric carbohydrates some of which have molecular weights of several millions. Examples are starch and glycogen. Polysaccharides are used extensively for medical, industrial, and nutritional purposes. These include their use as glues and adhesives, gelling agents, preparation of dyes and varnishes, anticoagulants and plasma volume extenders.

Monosaccharides are of great importance. Glucose in the body as sugar is utilised to provide several functions. The liver combines the blood glucose with other molecules to form the polysaccharide glycogen. This liver glycogen helps to maintain the blood glucose level. Muscles and other tissues remove glucose from blood to form glycogen which breaks down to provide energy to the tissues. Blood glucose serves as direct food for brain tissue.

Mammary glands combine glucose from the blood with galactose to form lactose which is milk sugar. Glucose is oxidised to provide more than half of the energy of the body. Monosaccharides can be modified to have structural alterations to perform specific functions of biological importance. Examples are the sugars deoxyribose and oxyribose in the nucleic acids DNA and RNA respectively. Other modified structures like amino sugars frequently occur in large quantities in structural material such as chitin of the exoskeleton of invertebrates, like crabs and lobsters. The monosaccharide fructose is rated as the sweetest sugar and is often the choice in the manufacture of candy.

In nature the most abundant disaccharides are sucrose and lactose. Sucrose, table sugar, occurs in plants where it is synthesized from D-glucose and D-fructose. The two sugar molecules are linked by a glycosidic bond.

Hydrolysis of sucrose by dilute hydrochloric acid (HCl) or by an enzyme invertase yields one molecule each of glucose and fructose. Sucrose is represented by the formula $C_{12}H_{22}O_{11}$ and has its structure as follows:

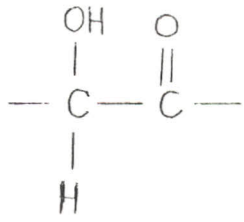


Sucrose

Sucrose is present in nectar of flowers, in juices of various plants. Ripe fruits are generally rich in sucrose. West, Todd, Mason and Van Bruggen (11) reported the annual production of sucrose, largely for food, as about 30,000,000 tons.

In foods, sucrose can be used as a sweetener. This property is largely due to the fructose component of the sucrose. It acts as a preservative in the food industry during canning, freezing and other processes. Sucrose helps in the caramelization of certain food preparations like cakes. Sucrose is a fermentable sugar and can therefore be used in the alcoholic beverages industry. During fermentation of sucrose by yeast, it is first split into glucose and fructose by invertase, and these monosaccharides are further fermented by other zymase enzymes.

As hydroxyaldehydes and hydroxyketones, most of the monosaccharides and disaccharides are effective reducing agents. This reducing action is utilised as a chemical basis for chemical test for sugars, e.g. to detect glucose in the urine of suspected diabetics. It can also be used to investigate sugars quantitatively and qualitatively. Reducing sugars act as reducing agents in alkaline solutions and they reduce oxidizing ions such as Copper ($^{2+}$). This property is used to determine the concentration of reducing sugars and sucrose. In the Lane-Eynon method for sugar determination a mixture of Fehlings solution A and B is used. Fehlings solution is composed of copper sulphate solution and alkaline tratrate solution. When the mixed Fehlings solution A and B is boiled with reducing sugars, the blue colour (from cupric tartar) is discharged and a red precipitate (cuprous oxide) forms. The bulk of the sugar solution is added first and then the remainder added dropwise. Since the end point is not clear, methylene blue (an internal redox indicator) is used towards the end of the titration and a slight excess of the sugar reduces the methylene blue to white, which can quickly be oxidized back to blue. In order to avoid being oxidised, the titration is performed as the solution being analysed is boiling to eliminate air. Heating time and temperature are controlled which otherwise affect results. Almost all monosaccharides are reducing sugars, because they contain free sugar groups in which the carbonyl group is part of an aldehyde or ketone group.



A free sugar group

Most disscharides are reducing sugars since one of the two anomeric carbons (reducing aldehyde or ketone group) in their structures is not in glycosidic linkage. Sucrose is not a reducing sugar because both of its anomeric carbons are in the bond that links the two monosaccharides. Other examples of non-reducing sugars include raffinose and trehalose.

Substances that rotate the plane of polarised light clockwise or to the right are said to be dextorotatory and they are designated D. On the other hand, those that rotate the plane of polarised light anticlockwise or to the left are levorotatory and designated L. Optical rotation phenomenon in carbon compounds is related to the presence of asymmetric carbon atoms in the molecule. When sucrose is hydrolyzed to yield one molecule each of glucose and fructose, the optical rotation changes from positive (+66.5°) to negative, (approximately -20°), because D-fructose is more levorotatory than D-glucose is dextrorotatory.

$C_{12}H_{22}O_{11}$	+	H_2O	⟶	$C_6H_{12}O_6$	+	$C_6H_{12}O_6$
+66.5°				+52.5°		-92°
Sucrose				D(+) glucose		D(-) fructose

(+ 52.5° - 92°) /2 = approximately - 20°

Such a reaction in which the sign of rotation is inverted is referred to as "inversion" and the mixture of glucose and fructose obtained is the "invert sugar". The ease with which the glycosidic bond of sucrose is hydrolyzed is related to the presence of the furanose ring.

The inversion of sugars can be used to determine the concentration of sucrose in fruits. The disaccharide sucrose is hydrolyzed and the concentrations of the reducing sugars glucose and

fructose are determined before and after inversion. The difference between the values is used to calculate sucrose content.

Fruits, especially when ripe, contain sucrose and reducing sugars. A fruit in the botanical meaning is a mature seed bearing structure of the flowering plant or according to Kays (7), a mature ovary that contains one or more seeds and may include accessory floral parts. Even though fruits lack a lot of nutrients, they are still of great importance nutritionally. They are soft fleshy parts of the plant and have fragrant aromatic flavours. Fruits fall under two groups based on their respiration during maturation and ripening. These are climacteric and nonclimacteric. Climacteric fruits are picked before ripening. Some tropical climacteric fruits are mango, papaya, banana and avocado. Nonclimacteric fruits are harvested at or after ripening. Some tropical examples are oranges and pineapples. The relative proportions of sucrose and reducing sugars vary from fruit to fruit, but most commonly the reducing sugars are present in greater amounts than sucrose. Traces of sugars like xylose, arabinose, galactose, mannose and maltose may be present in amounts which could be detected by chromatographic methods.

In Ghana, there are several groups of small scale co-operative fruits farmers who have already started exporting their produce to the international markets. These farms are situated in various parts of the country depending upon where the maximum yields are obtained.

a. Pineapples (Figure 1)

Pineapple is sometimes considered the best palatable fruit and called the King of Fruits. It belongs to the monocotyledon family Bromeliaceae. It is a nonclimacteric fruit. It is a herbaceous plant that is perennial (present at all seasons of the year) or biennial (grown vegetatively during the first year, it fruits and then dies during the second year). It is cultivated everywhere in the tropics and in some favoured parts of the subtropics, a special case being in green houses in the Azores (38 °N) (9a) . Sugar accumulates in the fruit during

the ripening process. Hulme (6a) reported the sucrose content as between 5.9 - 12.0% and the reducing sugars between 0.6 - 3.2%.

b. Mangoes (Figure 2)

Mango is a citrus fruit belonging to the dicotyledonous family Anacardiaceae. It is a climacteric fruit. It is fleshy drupe with edible pulp (mesocarp) and a woody stone (endocarp) around the seed. The size of the fruit varies from 3 to 38cm along the long axis and the weight from several grams to more than a kilogram. The colour of the ripe fruit is a mixture of green, yellow and red patches. Sucrose predominates in the ripe mango but reducing sugars increase rapidly at the expense of sucrose during the post climacteric period. Samson (9b) reported sugar content of mango as 15% and Hulme (6a) reported reducing sugars as 3.5%.

c. Oranges (Figure 3)

Orange belongs to the family Rutaceae. It is a nonclimacteric fruit. It is cultivated through out the subtropics and tropics roughly between 40° North and South latitude. Oranges possess fragrant aromatic flavors and are normally sweet or sweetened before eating. Simple sugars in oranges increase considerably to give sweetness during ripening. Different oranges have varied sugar contents. According to Samson (9c), the juice of a ripe orange or mandarin contains about 12% sugars, whilst Hulme (6a) reports a reducing sugar content of about 2.5%.

The objective of this project is to investigate the sucrose and reducing sugar content of pineapples, mangoes and oranges using qualitative, quantitative and chromatographic methods. Descending paper chromatography will be used to increase considerably the effective length of the run and thus improve separation.

Figure 1. Two Varieties of Pineapples

Note: A =*Ananas comosus* (Smooth Cayenne)

B = *Ananas comosus* (sugar loaf)

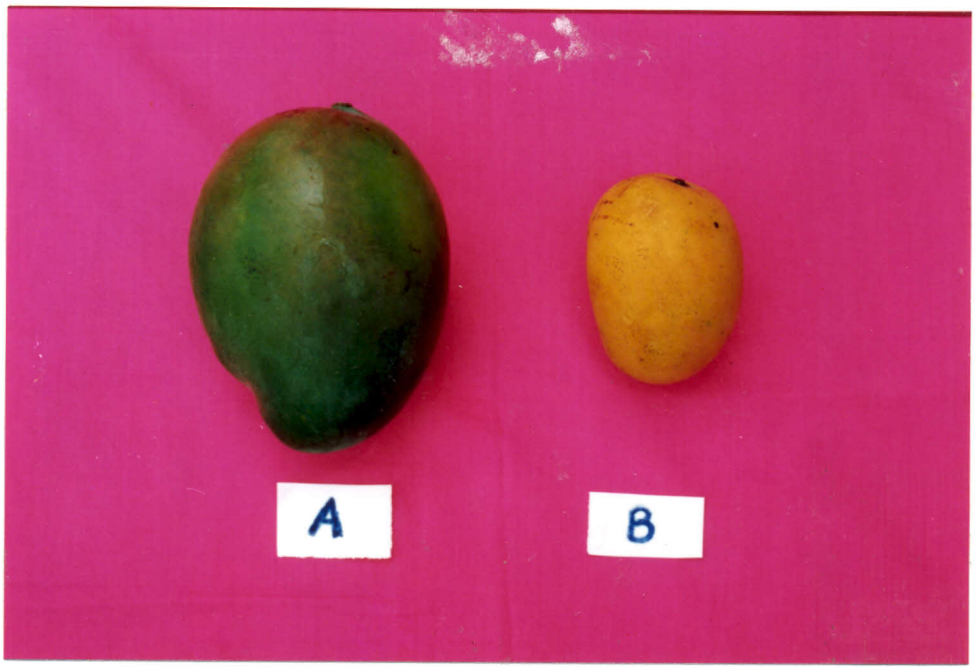


Figure 2 . Two Varieties of Mangoes

Note: A = *Mangifera indica* (Keitt variety)

B = *Mangifera indica* (Local seedling variety)

Figure 3. Two Varieties of Oranges

Note: A = *Citrus senensis* (sweet orange)

B = *Citrus reticulata* (mandarin)

CHAPTER TWO

MATERIALS AND METHODS

1. MATERIALS

A. Fruits

Two varieties each of the fruits were selected from some of the co-operative farms. The pineapples were obtained from the Central Region of Ghana . The mangoes and the oranges were from the greater Accra and Eastern Regions of Ghana respectively .

a.	Pineapples	(1)	*Ananas comosus* (Smooth Cayenne)
		(2)	*Ananas comosus* (sugar loaf)
b.	Mangoes	(1)	*Mangifera indica* (Keitt variety)
		(2)	*Mangifera indica* (Local seedling variety)
c.	Oranges	(1)	*Citrus senensis* (sweet orange)
		(2)	*Citrus reticulata* (mandarin)

B. Chemicals

The chemicals were purchased from two chemical companies:-

(a) FLUKA AG, CH- 9470 Buchs Switzerland.

D (+) Glucose, D (-) Fructose, D (+) Mannose, D (+) Maltose.

D (-) Ribose, D (+) Galactose, D (+) Sucrose, Invertase (Fructofuranosidase), Silver Nitrate, Sodium Acetate, Acetone, Acetic Acid, Methylene Blue

(b) BDH Chemicals Ltd., Poole, England.

Fehlings solution A and B, Sodium Hydroxide, Zinc Acetate, D (-) Arabinose, Iso-propanol, Ethyl Acetate, Ethanol, Hydrochloric Acid, Potassium Ferrocyanide, Ammonia solution

C. Filter Papers

These were obtained from Whatman International Ltd., Maidstone, England.

2. METHODS

A. Extraction of Fruit Juices

The fruits were thoroughly washed. In the case of the pineapples the crowns were removed before washing the fruits . The fruits were peeled and the juices squeezed out using a fruit squeezer. The juices were transferred into sample containers for the qualitative, quantitative and chromatographic analysis.

B. Qualitative Test

All tests were done in duplicate. Fresh Fehlings solution was prepared by mixing equal quantities of Fehlings solution A and B. To 2mls each of the extracted sample in the test-tube, 5mls of mixed Fehlings solution was added. The mixture was gently mixed and boiled for 5 minutes on a water bath. The samples were left to cool to room temperature and the colours developed observed with the naked eye.

C. Quantitative Analysis

a. Treatment of Fruit Juice

For each extracted fruit sample about 10g was accurately weighed into a 250ml beaker and 100mls of distilled water added. 5mls each of the clearing agents, zinc acetate and potassium ferrocyanide solution were added. The solutions were mixed using a magnetic stirrer and their pH adjusted to neutral with 50% NaOH. The contents of each beaker were quantitatively transferred into a 250mls volumetric flask and made up to the mark with distilled water. Each solution was filtered through Whatman No. 54 filter paper into a dry conical flask and stoppered.

b. Trial Titration

A trial titration was done to establish the right dilution, since initially, the reducing sugar contents of extracted juice samples were unknown. The trial titration was done as follows:
A 50ml burette (H - Bent type) was filled with the extracted test sample. 10mls of mixed Fehlings solution A and B was pipetted into a 250ml conical flask and 15mls of the extracted juice was added from the burette. The solution was boiled on a hot plate till the blue colour had nearly disappeared. Three drops of 1% methylene blue indicator were added and further 1ml aliquot of fruit extract was added to the boiling solution at 10 seconds intervals till the blue colour had disappeared. The volume of the burette reading was noted. In case the fruit solution was too concentrated, it was diluted and the dilution factor taken into account in the calculation. This was the case when after addition of the 15mls of the test solution, it changed rapidly to red on boiling. On the other hand fresh samples were prepared when the fruit sugar samples were too dilute. The concentration of the sugar solution should be such that the titration is between 15 - 50mls w/v sugar for 25mls Fehlings solution.

c. Standard Titration

Into a 250ml conical flask, 10mls of Fehlings mixed solution was pipetted, and 15mls of the friut extract was run into the flask from the burette before heating. The contents of the flask were mixed and gently heated to boil for 2 minutes. Three drops of the methylene blue indicator were added and the titration was completed within 3 minutes.

d. Total Sugars

i. Determination by Acid Hydrolysis

From each of the diluted clarified juice sample (250mls stock), 20mls was pipetted into a 100ml volumetric flask. About 30mls of distilled water was added followed by 5mls of concentrated acid (6M HCl). The flask was swirled round and immersed in a 70°C water bath for 10 minutes. The flask was cooled and neutralised with NaOH solution till a faint pink colour was obtained. The solution was made to the 100ml mark and titrated as above.

ii. Determination by Enzyme Hydrolysis

From each of the diluted clarified juice (250mls stock), 20mls was pipetted into a 100ml volumetric flask, and 30mls of distilled water added. 1.5% "invertase" w/v in 0.10M acetate buffer of pH 4.6 was set up alongside the juice extracts in a water bath and the flasks and samples were incubated for 15 minutes at 25^{o}C. To each sample, 5mls of the enzyme solution was added. The solutions were incubated for 10 hours. After 10 hours from the initial time, 10mls of 1% NaOH was added to stop the reaction. The flasks were removed, the solutions neutralized and titrated as above.

e. Invert Sugar Tables and Calculations

Standard invert sugar tables(8) for 10mls Fehlings solution was referred to in order to calculate the reducing sugar contents before and after inversion. (See Appendix 1 for Reference Tables). The percentage total reducing sugars was calculated before and after inversion using the formula:

% Reducing sugar = (Equivalent invert sugar x Volume x Dilution factor) / (Weight of sample taken)

The sucrose content was calculated by multiplying the difference between the reducing sugar contents before and after inversion by 0.95. Thus,

% Sucrose = (%Reducing sugar after inversion - %Reducing sugar before inversion) x 0.95

(see Appendix 2 for sample calculation).

D. Sugar Detection By Descending Paper Chromatography

Treatment of fruit juice

To 1ml of the juice sample, 3.0mls of 99%. ethanol was added. Using a bench centrifuge, the mixture was centrifuged for 5 minutes at 1000 x g. This was to remove salts and proteins

that could contaminate the sugars during development and detection of the chromatogram. The supernatant was decanted into sample tubes ready for spotting. For each of the following sugar standards, namely glucose, fructose, arabinose, maltose, sucrose, ribose, galactose and mannose, a 1% solution in 10% w/v isopropanol was prepared.

Preparation of Tank and Paper

The tank was presaturated with 100ml of the solvent system made up of ethyl acetate: acetic acid: water in the ratio 9:2:2. On each of two Whatman No. 2 chromatographic papers (dimentions 55 x 45cm), a line was drawn 7cm parallel to the edge. Another parallel line 3cm was drawn from the first line and on this second line, a number of small crosses were marked at equal distances apart. By means of a capillary tube a small drop each of the sugar standards as well as the fruit extracts were spotted on the crosses. The spots were dried with a hair dryer after each application. The spotted papers were hung from the glass trough (inert material) and an anchor was used to hold them. The papers were then passed over an anti-siphon rod slightly higher than the trough to prevent siphoning of the solvent. After pouring 200mls of the solvent into the trough, the greased lid was replaced tightly to prevent solvent evaporation. The papers were developed for 24 hours, removed and dried in a fume hood with a strong drought. By employing silver nitrate solutions, reducing substances were detected on the paper chromatograms. Saturated silver nitrate in acetone in an atomizer spray gun was used to spray the dried papers in a fume hood. The silver nitrate is reduced at the spots containing the reducing sugars and metalic sliver is deposited. The papers were quickly transferred into a tank saturated with 0.88M ammonia for 15 minutes to locate the spots. The papers were finally dried at 100°C for 15 minutes.

CHAPTER THREE

RESULTS

Figures 1, 2 and 3 show the three different fruits used namely, pineapples, mangoes and oranges. The two varieties of each fruit are also shown.

Qualitative Analysis

The qualitative analysis of all the fruits selected indicated the presence of sugar in them. A red precipitate was formed in each test, indicating that the blue colour from the cupric tartar had been reduced by the sugars to cuprous oxide (red precipitate). However, there were no noticeable differences in the colour intensities when viewed with the naked eye.

Quantitative Analysis

The contents of sucrose and reducing sugars were determined quantitatively before and after acid hydrolysis. Table 1 indicates that the sucrose content of the sugar loaf pineapple was higher than that of the Smooth Cayenne pineapple. The reducing sugar content was also higher. Table 2 indicates that the sucrose content of the Keitt mango variety was slightly higher than the local seedling variety. The reducing sugar content followed the same trend. Table 3 shows that, among the citrus species, mandarin had a very high sucrose content compared to that of the so called sweet orange. However, the reducing sugar content of both varieties were not very different. The observations made from Tables 1 - 3 show that the sucrose contents of the three different fruits namely pineapples, mangoes and oranges, varied considerably, the lowest amount of sucrose for sweet orange and the highest for mandarin, (from 4.9% -12.1%). The reducing sugar content of the fruits had a narrow range, the lowest amount for Smooth Cayenne pineapple and the highest for Keitt variety mango (2.1% - 4.9%).

Table 4 shows the reducing sugar content of the fruits after the extracts were hydrolysed with acid and enzyme. The comparison in this table indicates that the reducing sugars were higher

for enzyme hydrolysed samples than for acid hydrolysed samples. Table 5 shows that the sucrose content of the enzyme hydrolysed samples were higher than the acid hydrolysed samples.

Chromatographic Analysis

Figure 4 shows the separation achieved when the fruit samples were run alongside the sugar standards. The results (Table 6) indicate that all the fruits contained sucrose. In addition to sucrose, all the fruits contained fructose, glucose, arabinose and mannose. However, galactose, ribose and maltose were not found in any of the fruits.

TABLE I: AVERAGE SUGAR CONTENT OF PINEAPPLES

FRUIT VARIETY	REDUCING SUGAR CONTENT (% Fresh juice)		SUCROSE CONTENT (% Fresh juice)
	After Acid Hydrolysis	Before Acid Hydrolysis	
Smooth Cayenne	10.0	2.1	7.5
Sugar Loaf	12.8	3.1	9.2

TABLE 2: AVERAGE SUGAR CONTENT OF MANGOES

FRUIT VARIETY	REDUCING SUGAR CONTENT (% Fresh juice)		SUCROSE CONTENT (% Fresh juice)
	After Acid Hydrolysis	Before Acid Hydrolysis	
Keitt	15.6	4.9	10.2
Local Seedling	13.3	3.3	9.5

TABLE 3: AVERAGE SUGAR CONTENT OF ORANGES

FRUIT VARIETY	REDUCING SUGAR CONTENT (% Fresh juice)		SUCROSE CONTENT (% Fresh juice)
	After Acid Hydrolysis	Before Acid Hydrolysis	
Sweet Orange	9.6	4.4	4.9
Mandarin	16.8	4.1	12.1

TABLE 4: COMPARISON OF AVERAGE REDUCING SUGAR CONTENT OF DIFFERENT FRUITS (AFTER ACID AND ENZYME HYDROLYSIS)

FRUITS	REDUCING SUGAR CONTENT (% Fresh juice)	
	After Acid Hydrolysis	After Enzyme Hydrolysis
PINEAPPLES		
1. Smooth Cayenne	10.0	10.2
2. Sugar Loaf	12.8	12.9
MANGOES		
1. Keitt Variety	15.6	15.8
2. Local Seedling Variety	13.3	13.6
ORANGES		
1. Sweet Orange	9.6	9.8
2. Mandarin	16.8	16.8

TABLE 5: COMPARISON OF AVERAGE SUCROSE CONTENT IN DIFFERENT FRUITS ((AFTER ACID AND ENZYME HYDROLYSIS)

FRUITS	SUCROSE CONTENT (% Fresh Juice)	
	After Acid Hydrolysis	After Enzyme Hydrolysis
PINEAPPLES		
1. Smooth Cayenne	7.6	7.7
2. Sugar Loaf	9.2	9.3
MANGOES		
1. Keitt Variety	10.2	10.4
2. Local Seedling Variety	9.5	9.8
ORANGES		
1. Sweet Orange	4.9	5.1
2. Mandarin	12.1	12.1

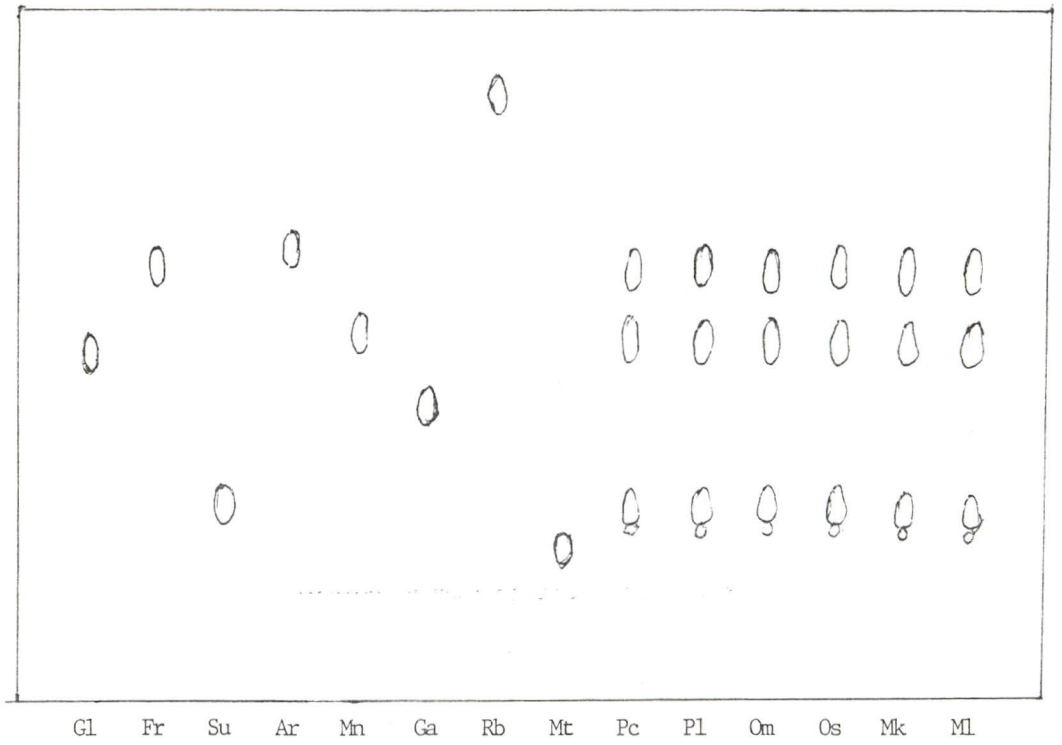
TABLE 6: SUCROSE AND REDUCING SUGARS FOUND IN DIFFERENT FRUITS

FRUITS	SUCROSE	FRUCTOSE	GLUCOSE	ARABINOSE	GALACTOSE	MANNOSE	RIBOSE	MALTOSE
Pineapple Smooth Cayenne	+	+	+	+	-	+	-	-
Pineapple sugar loaf	+	+	+	+	-	+	-	-
Mango Keitt variety	+	+	+	+	-	+	-	-
Mango local seedling	+	+	+	+	-	+	-	-
Sweet orange	+	+	+	+	-	+	-	-
Mandarin	+	+	+	+	-	+	-	-

Note: + indicates presence of sugar

\- indicates absence of sugar

Figure 4. One-way descending paper chromatographic separation of sugars in different fruits.



Note:

Standard sugars

Gl = glucose
Fr = fructose
Su = sucrose
Ar = arabinose
Mn = mannose
Ga = galactose
Rb = ribose
Mt = maltose

Fruit samples

Pc = pineapple Smooth Cayenne
Pl = pineapple sugar loaf
Om = mandarin
Os = sweet orange
Mk = mango Keitt variety
Ml = mango local variety

CHAPTER FOUR

DISCUSSION AND CONCLUSION

The quantitative results shown in Tables 1 - 3 show that sucrose was present in pineapples, mangoes and oranges. The results indicate that sucrose content varies from fruit to fruit but may not be all that significant among different varieties of the same fruit. Among the fruits selected, sweet orange contained the lowest amount of sucrose and mandarin the highest in the range (4.9% - 12.1%). The sucrose content of the Smooth Cayenne (7.2%) and the sugar loaf (9.2%) pineapples fall within the range reported by Hulme (1970). The sucrose contents of the green Keitt variety mango (10.2%) and of the yellow local seedling variety mango (9.5%) were fairly close but the sugar content of the local seedling variety was lower than 15%. This has also been reported by Samson (1986). Both the green and yellow mangoes were matured and ripe and the extent of ripeness might have contributed to the slightly lower sugar content of local seedling variety (less than 15%). This is because the sucrose content of mangoes increase during ripening but tend to decrease slightly during post climacteric period. Samson (1986) reported the sugar content of orange and mandarin to be about 12%. The results show that the sucrose content of mandarin was 12.1% but that of sweet orange was 4.9%. The latter was unexpected. The mandarin was collected from the farm at normal harvest, and even though it was expected to be matured, the extent of maturity as well as climatic and other conditions might have contributed to such low sucrose content.

The reducing sugar content of all the different fruits as well as their varieties were in the range 2.1% - 4.9%, with Smooth Cayenne pineapple having the lowest and the Keitt variety mango the highest. These values of the reducing sugars are similar to those reported in literature

For the chromatographic analysis for detection of sugars in the fruits, two solvent systems were used. The first solvent, ethyl acetate: iso-propanol: water in the ratio 6:2:2, resulted in poor separations of the sugars. The separations were better using the solvent ethyl acetate: acetic acid: water in the ratio 9:2:2. Figure 4 shows that sugars in the three different fruits

followed the same pattern of separations. Table 6 shows that sucrose, glucose fructose, mannose and arabinose were detected in pineapples, mangoes and oranges. Galactose, ribose and maltose were not detected in any of the fruits.

Some researchers found sucrose, fructose and glucose in several fruits and that fructose and glucose are the main reducing sugars in fruits (Hulme 1970). The report by Hulme (1970) indicates also that mannose had been detected in oranges and arabinose in mangoes. Galactose has been reported to be present in some fruits but its presence is in traces which could be detected by gas chromatographic methods.

The varied sucrose and reducing sugar content of different fruits contribute significantly to differences in their sweetness. This could be an important factor for choosing fruits for commercial, industrial and domestic uses. It will be interesting to carry out a study in which the sucrose and reducing sugar content of several varieties of the same fruit are studied.

APPENDIX 1

INVERT SUGAR TABLES

Invert Sugar Table for 10 ml Fehling's Solution

ml of sugar solution required	Solutions containing besides invert sugar: No sucrose		1 g sucrose per 100 ml		5 g sucrose per 100 ml		10 g sucrose per 100 ml		25 g sucrose per 100 ml	
	Invert sugar factor*	mg invert sugar per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml
15	50·5	336	49·9	333	47·6	317	46·1	307	43·4	289
16	50·6	316	50·0	312	47·6	297	46·1	288	43·4	271
17	50·7	298	50·1	295	47·6	280	46·1	271	43·4	255
18	50·8	282	50·1	278	47·6	264	46·1	256	43·3	240
19	50·8	267	50·2	264	47·6	250	46·1	243	43·3	227
20	50·9	254·5	50·2	251·0	47·6	238·0	46·1	230·5	43·2	216
21	51·0	242·9	50·2	239·0	47·6	226·7	46·1	219·5	43·2	206
22	51·0	231·8	50·3	228·2	47·6	216·4	46·1	209·5	43·1	196
23	51·1	222·2	50·3	218·7	47·6	207·0	46·1	200·4	43·0	187
24	51·2	213·3	50·3	209·8	47·6	198·3	46·1	192·1	42·9	179
25	51·2	204·8	50·4	201·6	47·6	190·4	46·0	184·0	42·8	171
26	51·3	197·4	50·4	193·8	47·6	183·1	46·0	176·9	42·8	164
27	51·4	190·4	50·4	186·7	47·6	176·4	46·0	170·4	42·7	158
28	51·4	183·7	50·5	180·2	47·7	170·3	46·0	164·3	42·7	152
29	51·5	177·6	50·5	174·1	47·7	164·5	46·0	158·6	42·6	147
30	51·5	171·7	50·5	168·3	47·7	159·0	46·0	153·3	42·5	142
31	51·6	166·3	50·6	163·1	47·7	153·9	45·9	148·1	42·5	137
32	51·6	161·2	50·6	158·1	47·7	149·1	45·9	143·4	42·4	132
33	51·7	156·6	50·6	153·3	47·7	144·5	45·9	139·1	42·3	128
34	51·7	152·2	50·6	148·9	47·7	140·3	45·8	134·9	42·2	124
35	51·8	147·9	50·7	144·7	47·7	136·3	45·8	130·9	42·2	121
36	51·8	143·9	50·7	140·7	47·7	132·5	45·8	127·1	42·1	117
37	51·9	140·2	50·7	137·0	47·7	128·9	45·7	123·5	42·0	114
38	51·9	136·6	50·7	133·5	47·7	125·5	45·7	120·3	42·0	111
39	52·0	133·3	50·8	130·2	47·7	122·3	45·7	117·1	41·9	107
40	52·0	130·1	50·8	127·0	47·7	119·2	45·6	114·1	41·8	104
41	52·1	127·1	50·8	123·9	47·7	116·3	45·6	111·2	41·8	102
42	52·1	124·2	50·8	121·0	47·7	113·5	45·6	108·5	41·7	99
43	52·2	121·4	50·8	118·2	47·7	110·9	45·5	105·8	41·6	97
44	52·2	118·7	50·9	115·6	47·7	108·4	45·5	103·4	41·5	94
45	52·3	116·1	50·9	113·1	47·7	106·0	45·4	101·0	41·4	92
46	52·3	113·7	50·9	110·6	47·7	103·7	45·4	98·7	41·4	90
47	52·4	111·4	50·9	108·2	47·7	101·5	45·3	96·4	41·3	88
48	52·4	109·2	50·9	106·0	47·7	99·4	45·3	94·3	41·2	86
49	52·5	107·1	51·0	104·0	47·7	97·4	45·2	92·3	41·1	84
50	52·5	105·1	51·0	102·0	47·7	95·4	45·2	90·4	41·0	82

* mg of invert sugar corresponding to 10 ml of Fehling's solution.

APPENDIX 2

SAMPLE CACLULATION

Sample used : Mango Keitt variety

A. Total reducing sugars (after acid hydrolysis)

Weight of sample taken	=	10.17g
Sample diluted to volume	=	250mls
Dilution factor: 20mls sample	=	5
diluted to 100mls		
Volume of Fehlings solution used	=	10.0mls
Titre value (mean)	=	41.0mls
Reference to standard table, 41.0mls	=	127.1mg
	=	0.1271g invert sugar per 100g
% Total reducing sugar	=	(0.1271 x 250 x 5) /10.17
	=	15.6%

B. Reducing sugars (before acid hydrolysis)

Wt. of sample taken	=	10.17g
Sample diluted to volume	=	250mls
Dilution factor	=	No further dilution
Volume of Fehlings solution	=	10.0mls
Titre value (mean)	=	26.0mls
Reference to standard table, 26.0mls	=	197.4mg
	=	0.1974g invert sugar per 100g
% Reducing sugars	=	(0.1974 x 250) /10.17
	=	4.9%

C. Percent Sucrose

% Sucrose = (%Total reducing sugars after inversion - % Total reducing sugars before inversion) x 0.95

= (15.6 - 4.9) 0.95

= 10.2%

APPENDIX 3

PREPARATION OF CHEMICALS

A. Clarifying Reagents

a. Potassium ferrocyanide solution. 10.6% aqueous solution - 100mls

10.6g potassium ferrocyanide was weighed and dissolved in 100mls distilled water.

b. Zinc acetate. 21.9g crystallised zinc acetate was weighed and added to 3mls glacial acetic acid and solution made up to 100ml with distilled water.

c. 5mls each of solutions 1a and 1b form the clarifying reagent.

B. Sodium hydroxide solution. 50% w/v - 200mls

100g sodium hydroxide pellets was dissolved and made up to 200mls with distilled water. The solution was cooled before use.

C. Methylene blue solution. 1% - 50mls

0.5g methylene blue was dissolved and made up to 50mls with distilled water.

D. Fehlings solutions A and B (Commercially prepared)

Equal volumes of solutions A and B were mixed fresh for use.

E. Iso - propanol. 10% v/v - 100mls

10mls iso - propanol was made up to 100mls with distilled water.

F. Standard sugar solutions. 1% w/v/v - 1ml

0.01g each of the sugar standards was dissolved in 1ml of 10% v/v iso-propanol.

G. Detecting reagent

a. Saturated silver nitrate. 5g silver nitrate was dissolved in 3ml water.

b. 3ml saturated silver nitrate solution was added to 60ml acetone and few drops of distilled water were added till precipitate just dissolved.

H. Chromatographic solvent system -1litre.

Ethyl acetate: acetic acid: water (9:2:2). 692mls ethyl acetate, 154mls glacial acetic acid and 154mls distilled water were mixed together.

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