THE CHEMICAL ANALYSIS OF SUGARS AND ACIDS IN GUAVA JUICE FROM VARIETIES GROWN IN KENYA

By

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This thesis is my original work and has not been presented for a degree in any other University.

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1. INTRODUCTION

Guava is one of the important pomiferous fruits of the Myrtle family. The species Psidium Guajava L. is native to South America and widely distributed in the southern tropic. The fruit has been introduced into West and South Africa and several of the Pacific Islands (22).

In Kenya it grows wild in the Machakos area, part of the Nyanza, Western provinces and the Coast province. The three varieties found in Kenya are Marherber-pink flesh, Patnangola - white flesh, and Hawaiin hybrid - light pink flesh. The fruit weight ranges from 50 - 100 gm. The most common post-harvest disease is anthracnose caused by Colletotrichum psidii, with maximum development at about 30^{o}C and 96% Relative Humidity (11).

Guava tree grows well on land which is considered marginal for other crops. Therefore there are many areas in Kenya where it would be feasible and profitable to grow the fruit as cultivated orchard crop.

The knowledge of the chemical composition of the fruit, and in particular the sugars and acids, which impart important properties to the derived products, is of great value to processors and for consumer acceptance

Ascorbic acid (Vitamin C) content of guava is a very good asset, and the fruit will become more and more important as the public learns the high value of guava products in supplying this essential food nutrient.

Conflicting reports of the non-volatile acids in guava are also of great interest. A few workers (8) have reported the presence of lactic acid in guava, which is a product of fermentation, and not usually present in fruit juices except in wines and juices made of raspberry (35).

The above therefore warrants the necessity to know the exact composition of guava and also of guava grown in Kenya for potential processing. Although the industrial use of guava is more and more recognised it has not yet been intensively used in Kenya and elsewhere.

2. LITERATURE REVIEW

2.1. Guava Products

For the production of guava products, firm yellow and mature fruits with no sign of insect or fungus damages are chosen. Half ripe fruits are stored at $2^{o}C$ - $7^{o}C$ and allowed to ripen (30). Green fruits are not good for processing as they do not have full flavour nor do they yield as much product as the fully mature fruit. In view of the increasing importance of guava for processing some studies have been done to find optimum storage conditions for the harvested fruit (12).

In Hawaii, the guava processors state that for puree preparation the fruit should have a good colour, flavour, few seeds, small cells, a pH range from 3.3 - 3.5 and a soluble solids content of 9 - 12%. Large fruits with thick flesh are preferred. Firm but fully ripe fruits are required (34).

Simple methods are available to evaluate the colour and the texture. The use of guavas of poor colour and texture for processing will do more harm to consumer acceptance of the final products than any other factors which a processor can control. It is therefore desirable to check the products for these two quality attributes on all consignments of fruits.

Guava fruit is consumed mainly in the processed form in a few developed countries, but is eaten fresh or has found local use in many tropical parts of the world. The processed forms of guava include:-

(1) puree

(2) clarified and cloudy juice

(3) nectar

(4) powder

(5) jelly

(6) ketchup

(7) cheese (34).

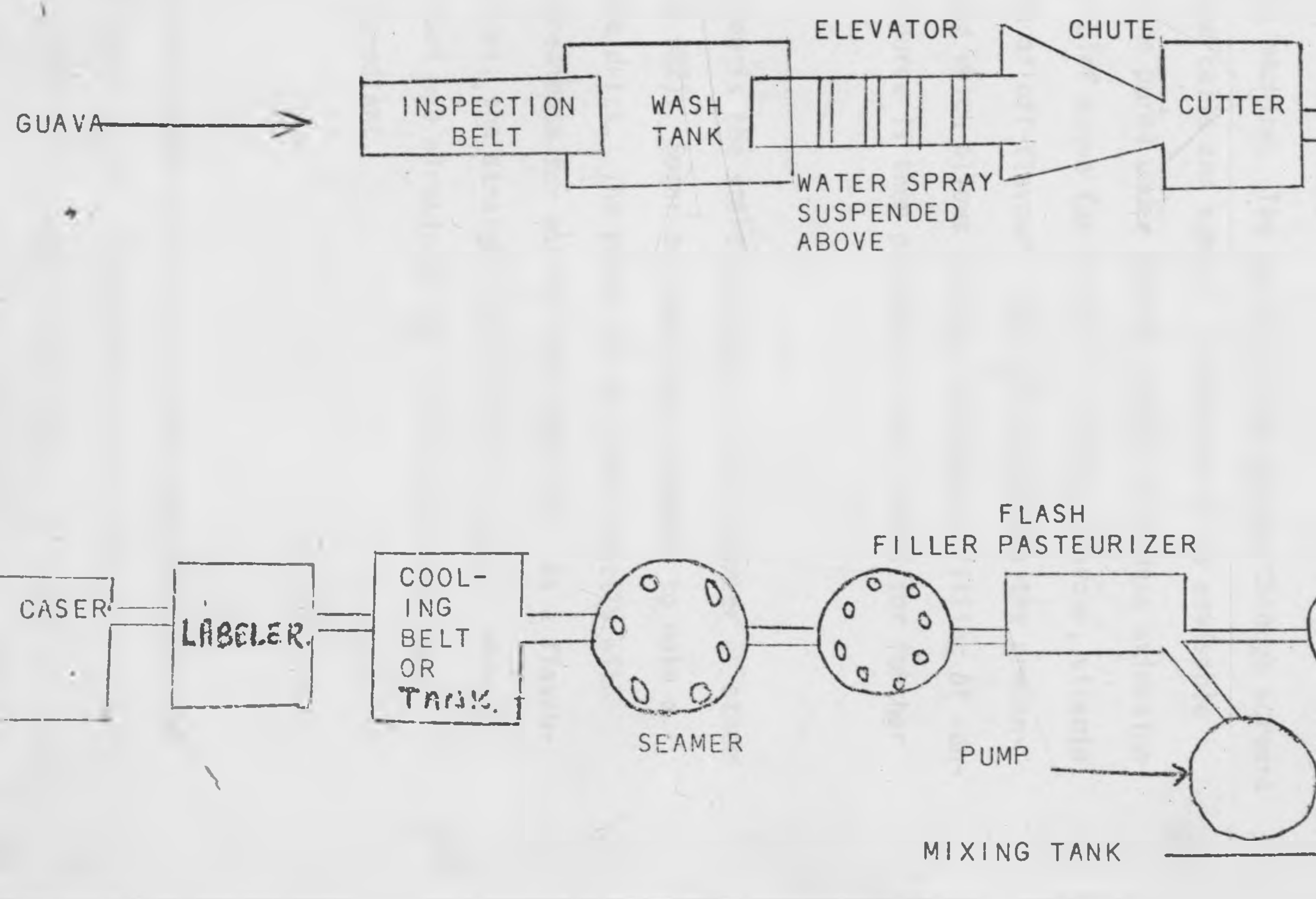
Table 1 shows the criteria for quality measurement of fruits from seedlings of selected Hawaiin guavas, and Figure 1 is a flow sheet for a guava processing line.

Table I: Quality Measurement of Fruit from selected Hawaii

dling	Fruit Tested	Diameter (cm)	Weight (gm)	% Seed	Colour	Flavour	Soluble solids (%)	pH
30	11	6.35-7.62	227.3	1.6	Strong pink	Normal mild sour	8.0	3.5
31	13	6.35-7.62	170.1	3.7	Light pink	Normal sour	11.5	3.2
-1	3	5.08-6.35	99.2	4.1	Strong pink	Normal slightly sweet	10.6	4.0
pi 1	6	6.99	178.6	4.4	Mild pink	Mild slightly sour	9.8	3.7

Source Boyle et al and reproduced by Luh (22

FIG. 1: FLOW SHEET FOR A GUAVA PROCESSING LINE (BOYLE ET AL. RE-PRODU



Guava Puree

Guava is said to be one of the easiest fruits to process.

The whole fruit can be fed into a paddle pulper for maceration into puree. If the fruits are rather firm, it may be necessary to attach a chopper or slicer to the hopper which feeds into the machine. The puree is then passed through screens to remove stone cells and seeds. Afterwards it is advisable to deaerate the puree under vacuum, mainly to reduce oxidation which is the chief cause for losses in colour, flavour, vitamin, and production of off-flavour. The third point is the prevention of foaming which allows correct and uniform filling of containers. The puree is then pasteurized and stored for further usage.

Guava puree is the starting material for a number of other guava products (22). Sugar and water may be added to make a nectar or juice drink. The puree can be used directly with commercial ingredients for making ice-cream etc. As a flavouring for pastries, the straight guava puree is used. Several fruit punch bases are already on the market which use guava puree as an ingredient.

Guava Nectar

The puree mentioned above is the basic ingredient for the production of guava nectar. Depending on the content of soluble solids of the puree (22), proportional amounts of sugar and water are added to make the final product with a specified soluble solids content. The soluble solids of the puree obtained from each batch of fruit is measured in order to control the final soluble solids of the nectar.

The following formulation (22) for a nectar having 20% puree by weight shows the amount of ingredients to be mixed to have a finished product of approximately 11% soluble solids and a pH of 3 - 4.

Guava puree (soluble solids 7%)	100 Kg.
Cane Sugar	48 Kg.
Water	352 Kg.
Yield of guava nectar	500 Kg.

All the components are mixed in stainless steel kettles or tanks. The mixture is passed through a heat exchanger at a temperature of 82°C - 87°C and held for 60 seconds. The product is filled hot into lacquered cans. The cans are sealed, inverted for three minutes, and cooled with water to about 37°C.

Some processors believe that the addition of a small amount of acid such as citric enhances the natural flavour of the product. Sodium benzoate may be added as a preservative up to the limit allowed by the food regulations, but is usually not necessary.

<u>Guava Juice</u>

The production of guava juice cannot be achieved by conventional methods of juice extraction. Three methods are used at present: the freezing method, the heat treatment and the enzyme treatment.

In the first method whole guava fruits are frozen to help break their internal structure and are kept in frozen form until needed. This method seems expensive because the processor needs large cold storage rooms to keep the frozen fruits. When whole guavas are used the yield of juice is less due to interference of the seeds in the pressing out of the juice (22).

In the second method, as used by one Kenyan firm, partially sliced fruits are heated to about 60^{0}C for 10 to 15 minutes to break down the tissues. It seems that a good yield can be obtained, but it is clear that this method may reduce considerably the ascorbic acid content and the flavour.

In the third method an enzymatic treatment of the pulped guava fruits is used to hydrolyse the pectin. It seems that a maximum juice yield of 85% can be obtained by using ripe guava fruit. Different commercial pectolytic enzymes giving different yeilds may be used for this purpose (24). The enzymatic treatment of guava seems to be so far the most economic method. However, depending on the temperature used during the mash treatment, the flavour of the juice may be affected. A lower reaction temperature would be more advisable. However, the exact optimal conditions of temperature and time have to be determined in preliminary experiments.

The guava juice can be used as single strength juice or as a concentrate. Some manufacturers prefer to blend the guava juice with other fruit juices or extracts to retain the nice guava flavour but not the colour of the fruit.

Guava Pectin

A recent study (26) has shown that guavas grown in Kenya contain an average of 11% of pectin on dry matter basis and that the fruit may be used for the direct production of low methoxyl pectin. Muroki (25) showed that to obtain a gel of 60 ^{o}Brix at a pH of 2.90 the addition of 0.53 - 0.69% guava pectin was necessary.

2.2. The chemical composition of guava

The chemical composition of the fruit depends largely on the combined influences of genetic regulatory mechanism and environmental conditions to which the growing fruits are subjected during their development. The post harvest handling can also affect the chemical composition of the fruit (30).

Depending on the guavas analysed, different compositions have been reported as shown in tables 2, 3, and 4.

Table 2: The chemical composition of guava by Benk (3).

Water	78 - 83%
Total sugar	3 - 11%
Protein	0.7 - 1.0%
Fat	0.4 - 0.6%
Total acidity	0.36%
Pectin	0.3%
Minerals	0.21%
Phosphate	10.9mg/100gm
Sodium	1.9mg/100gm
Calcium	3.0mg/100gm
Potassium	14.5mg/100gm
Vitamin C (ascorbic acid)	35.7mg/100mg

Table 3: The chemical composition of guava by Osborn (29)

	*Total Sugars invert (%)	Mineral Content (%)	Soluble Solids in ^{o}Brix
Maximum	10.0	0.86	16.3
Minimum	3.30	0.46	6.4
Average	5.71	0.62	9.8
Std. deviation	2.45	0.16	3.8
No. samples	6	6	6

* The sugars were calculated as percent fresh weight.

Table 4: The mineral content of guava by Jacobs (19)

Average	0.7%
Maximum	1.0%
Minimum	0.46%

The acids and sugars, important constituents of the guava, are dealt with in the chapters 2.2.1. and 2.2.2.

The mineral content of guava as reported by Benk (3), Osborn (29), and Jacobs (19) ranges from 0.2 to 1.0%. These variation of the mineral content is influenced mostly by the variety and the type of soil on which the fruits were grown and to a lesser extent on the climatic conditions. The average mineral content reported by Jacobs is 0.7% while Osborn reported 0.62%. Both workers found the same minimum content of 0.46%. Benk (3) determined a lower mineral content of 0.21%. This is probably due to the above mentioned reasons, and perhaps to the type of analytical method employed.

2.2.1. Acids

Lodh and Pantastico (21) found that titratable acidity in guava increased smoothly and rapidly from fruit set to fruit ripening. Ascorbic acid followed a similar trend. From a practical point of view the titratable acidity has been used as an indicator of the stage of maturity of guava and an objective information relating to flavour.

The acid components of the fruit impart important properties to the food products, being mostly prominent in flavour, processing and preservation (8). Addition of edible organic acids such as citric, malic or fumaric is commonly employed to lower the pH and to influence the flavour.

The presence of a particular acid can be used as a criterion of adulteration of fruit products. Santini (36) reported that Gumarals and Maria de Abreau, working with guava products, did not find citric acid present in the fruit, and they suggested that this lack could be used as a test for adulteration of jellies; the presence of citric acid in guava products could then be regarded as adulteration. Since in Gumarals and Abreu's paper there is no reference to the analytical method employed or to the guava varieties used, Santini (36) decided to repeat this work quantitatively using different varieties of guava. He found citric acid to be the predominant polybasic organic acid in guava. This has been later confirmed (37). Therefore its presence cannot be used as a test for adulteration of guava products.

Santini (36) also found tartaric acid as well as L-malic acid in small amounts in the three varieties of guava analysed. Chan et al. (8) showed the presence of six organic acids, five of them identified as lactic, malic, citric, ascorbic, and galacturonic acid and assumed the presence of tartaric acid.

It is well known that guava is an excellent source of ascorbic acid ranging well over 50 mg/100 gm (8). The peel is reported to contain more ascorbic acid than the flesh (13). Santini (36) reports the total average ascorbic acid including dehydro-ascorbic acid in the Dominica Roja Agria variety of guava as 230 mg/100 mg. The average ascorbic acid content reported by Ulrich (43) is 300 mg/100 gm, Benk (3) gives a figure as low as 35 mg/100 gm. El Faki and Saeed (12) reports 203 mg/100 gm in the peel and 96 mg/100 gm in the pulp of white guavas, and for pink guavas the figures are 118 mg/100 gm and 49 mg/100 gm respectively. It appears that the ascorbic acid content of guavas varies widely not only with the variety but also with the origin of the fruit. In determining the ascorbic acid content, it is advisable to keep in mind that both the environmental conditions during growing and the storage conditions affect the content of ascorbic acid in the fruit.

The ascorbic acid is easily oxidized into dehydro-L-ascorbic acid by enzymes when the cellular structure is destroyed by mechanical damage, rot or senescence (23). The enzymes that occur in fruit and which may be responsible for oxidative destruction of the acid are: ascorbic acid oxidase, phenolase, cytochrome oxidase and peroxidase. There is evidence that many fruits contain substances which inhibit the oxidative activities of these enzymes.

Mapson (23) thinks Somoygi was the first who had reported the existence of substances which inhibited the oxidation of ascorbic acid by phenolase, peroxidase, ascorbic acid oxidase or by cupric ion.

Ascorbic acid stability in fruit juices is reported (23) to be due to the presence of polybasic or polyhydroxy acids such as citric acid and malic acid which are also present in large quantities in guava juice. These acids by virtue of their power to chelate metal ions reduce the catalytic activity of metals like copper and iron. Sugars due to their concentration in fruit juices and their tendency to chelate metal ions may also contribute to some extent to the stability of the ascorbic acid (23).

As a result of the stability of ascorbic acid in fruit juices only small losses are usually encountered during actual processing. Thus for example in jelly manufacture the chief loss occurs if the harvested fruit is allowed to become overripe, or if damaged by mechanical means and is held in this condition for any length of time before processing (23).

Lactic acid is not usually present in fruit juices. This follows from the known fact that lactic acid and succinic acid are normally products of alcoholic fermentation. Ryan and Dupont (35) found lactic acid in all wines examined, but in all other juices investigated they found lactic acid in raspberry only. Chan et al. (8) identified lactic acid in guava by thin layer chromatography and determined its amount by gas liquid chromatography to be in the order of 0.25% for the Beaumont variety and 0.012% for wild guavas. The conflicting reports of the presence or absence of lactic acid in juices and especially in guava juice provides an area which still requires further investigation.

Different chromatographic methods can be used for the quantitative determination of acids in foods and food products.

Column chromatography has been applied by Bulen et al. (7) for the separation and identification of acids in plant tissues. The method reported by Bulen et al. (7) uses an initial separation on a silica gel column, followed if necessary, by additional separation on other columns with different solvents. One disadvantage of the method is that it is very tedious and therefore is seldom used.

The accurate quantitation of acids by paper and thin layer chromatography is possible provided precautions are taken. These methods are used in laboratories where gas liquid chromatography which seems to be more reliable is not available. Quantitation may be done using spot areas measurement, densitometry, or the technique of elution (38).

Gas liquid chromatography is the most recent procedure for the separation, identification, and quantitation of common fruit sugars and organic acids. The method involves extraction and purification by ion-exchange chromatographic procedures. The acids are then converted into their trimethylsilyl derivatives for analysis by gas liquid chromatography (6, 8, 10, 12, 14, 15, 16, 17, 31, 35).

2.2.2. Sugars

Sugar content of fruits varies considerably with the soil and climatic conditions during the development of the fruit. In addition changes in sugar content can occur before full ripeness. Rodrigues et al. (34) found a decrease in total sugar content of guava fruit during the last ten days of fruit maturation. Lodh and Pantastico reported that total and reducing sugars increased during growth (21).

The most abundant sugars in fruits are fructose, glucose and sucrose. Sucrose is the main dissacharide present in fruits. Its concentration is often determined by the differences of the reducing sugar content before and after inversion. Other sugars are rarely present, and when they are, only in small amounts. Of the minor sugars arabinose has been detected in guava (44).

Of the sugar derivatives, unripe guava has been reported to contain 0.1% of an unusual sugar ester which completely disappears as the fruit ripens (44). The two cis-hydroxyl group attached to carbon-three and-four of the L-arabinose are esterified with two hexahydroxy-diphenic acid.

The taste of a fruit or fruit product is greatly determined by the balance between sugars and acids. The sugar acid content of guava is therefore of special importance to the consumer and the processors. A very high sugar content of fruits is usually not recommended to processors, as this could lead to browning reactions in the fruit products.

The determination of the soluble solids content is important to the commercial fruit processors and has been chosen as one of the criteria for good guava fruits and products. Processors prefer fruits with a soluble solids content of 9 - 12% (34). Beyond this the fruit is considered to be too sweet for processing. The determination of soluble solids content is also important in formulation of different products and in blending of different batches to arrive at a standardized finished product.

3. Materials and methods

3.1. Guava

Three varieties of guava were obtained from the National Horticultural Research Station at Thika. Wild guavas were bought in the market.

The varieties of guava obtained were as follows:-

Patnangola	- white flesh
Marherber	- pink flesh
Hawaiin hybrid	- light pink flesh

3.2. Chemicals and materials

Laboratory grade chemicals were all purchased from Howse and McGeorge Limited, Nairobi, Kenya.

The pectolytic enzyme, Pectinex, super concentrate, was provided by the Schweizerische Ferment AG, Basel, Switzerland.

Amerlite IR - 120 (H) cation resin and Permutit Iso por De-Acidite FF-IP(HO) anion resin were both purchased from Howse and McGeorge Limited, Nairobi.

Precoated thin layer plates with cellulose and silica gel were obtained from Camag, Switzerland.

The acid and sugar standards for thin layer and paper chromatography were purchased from Howse and McGeorge Limited, Nairobi.

The following acid standard were used:

Lactic acid
Succinic acid
Malic acid
Tartaric acid
Galacturonic acid
Ascorbic acid
Citric acid

The following sugar standards were used:

Glucose

Fructose

Sucrose

Arabinose

3.3. Methods

3.3.1. Preparation of guava juice

The fruits were washed thoroughly and any infected fruits discarded. 1-4 kilogram of guava fruits were macerated using a waring blender. The puree obtained was stored in frozen form in plastic containers prior to further processing. The puree was thawed and completely macerated using a pectolytic enzyme, Pectinex super concentrate. Since the yield was not of major importance in these experiments, the amount of 50 grams Pectinex super concentrate per 100 kilograms recommended by the manufacturer was used. The mixture was stirred in a water bath at 45-55°C for 20 - 30 minutes.

The pectolytic enzyme hydrolises the pectic substances and releases the juice more easily. The half clarified juice was pressed through a cheese cloth using a simple stainless steel hand operated press (25). The juice was stored in bottles in the deep freezer (-18°C) till further analysis. For the purification of the acids the ion - exchange technique by Heartbell (16, 17) was used.

3.3.2. Determination of acids

pH and titratable acidity

pH measurement of the juice was carried out using a Pye Unicam Model 290 MK 2 pH meter.

For the determination of titratable acidity the A.O.A.C. method (1) has been used, taking 10ml of juice and titrating with NaOH 0.1N to pH 8.2.

Determination of volatile acids

For the determination of volatile acids the method of the International Federation of Fruit Juice Producers was used (18).

Determination of the non-volatile organic acids

Preliminary experiments showed the paper chromatography to be better in organic acid identification. Therefore the final experiments were done using paper chromatography.

The method used was according to (8) and (33). The solvent chosen for development was as follows: benzyl alcohol: tert. butyl alcohol: isopropyl alcohol: formic acid: water (B.B.I.F.W.), (28: 8: 8: 1: 8v/v).

Table 5 shows the methods used for quantitative determination of five acids.

Table 5: Methods used for quantitative determination of five acids.

Acids	Quantitative method of determination	References
Citric acid	Paper chromatography	(8, 33)
Malic acid	" "	(8, 33)
Tartaric acid	paper chromatography	(8, 33)
	& chemical analysis	(18)
Lactic acid	chemical analysis	(18)
Ascorbic acid	chemical analysis	(2)

The concentration of the reference acids used ranged from 1mg/100ml - 9mg/100ml. The descending technique was used for development.

The acid spots were located using bromocresol green 0.4gm in 100ml of 95% aethanol solution adjusted to pH 5.5 using 0.1 N NaOH.

Three acids which appeared in abundance, citric, malic and tartaric, were then quantitatively determined using spot area measurement on the paper chromatograms.

After the acids had been located on the paper chromatogram, the area of the spots was determined by laying a sheet of transparent paper on the chromatogram tracing the outline of the spots, and measuring the area of the drawing by super-imposing the tracings on millimeter graph papers.

A standard curve was drawn for each acid by plotting areas versus concentrations and the sample concentration read from the curve.

Lactic acid and tartaric acid were determined using the methods of the International Federation of Fruit Juice Producers (18).

The method described by Barakat et al. (2) was used for the determination of the ascorbic acid. This method was preferred to the method which uses dichlorophenol indophenol because the end point was easier to determine especially with the Marheber and the Hawaiin hybrid varieties.

3.3.3. Determination of sugars

The soluble solids contents expressed as ^{o}Brix were determined using a hand refractometer, (Kikuchi Tokyo. No. 75027) W.S.R. The invert sugars and total sugars were determined according to Rentschler and Tanner (33). The paper chromatographic method of Rentschler and Tanner (33) was used for qualitative determination of sugars but the following solvent for development was preferred.

n-butanol: pyridine: water (75: 15: 10 v/v).
The location reagent was 3gm p-anisidine hydrochloride in 95ml n-butanol and 5ml water.

The standard mixture consisted of glucose, fructose, arabinose and sucrose.

3.3.4. Determination of mineral contents

The mineral content was determined as ash using the A.O.A.C. method (1).

4. Results and Discussion

4.1. Acidity

Below in Table 6 are the results of the analysis for acidity expressed as pH and the titratable acidity calculated as citric acid. The volatile acids were calculated as acetic acid. The analysis were done using unclarified juice.

Table 6: The acidity expressed as pH, titratable acidity, volatile acids.

Varieties	pH	Titratable acidity (calculated as citric acid)	Volatile acids (calculated as acetic acid)
Hawaiin hybrid	3.6	1.49g/100ml	24mg/100ml
Pink guava	3.6	1.15g/100ml	34mg/100ml
White guava	3.7	0.45g/100ml	60mg/100ml

The figures are the average of four samples analysed per variety.

pH

The pH of the three varieties are almost the same. These values have to be expected since the pH of most fruit juices usually ranges between 2 - 4.

Titratable acidity

The results in Table 6 show the white guava has the lowest titratable acidity. The Hawaiin hybrid has the highest percentage of titratable acidity. The white guava is the least sour and the Hawaiin hybrid considered most sour.

In the literature average titratable acidity for guava has been quoted 0.87% calculated as citric acid (43). Since titratable acidity changes with growth, it is not easy to determine a definite figure. Some investigators (43) have proposed titratable acidity to be used as an index of maturity. It is true that titratable acidity may give some information concerning the stage of maturity, but it seems important to have a better knowledge of the different acids actually present in the fruits. It would appear from these results that guavas growing in Kenya have relatively high acid content. That explains why it was necessary to add sugar to the juice made in our laboratory from pink guavas in order to have an acceptable product and to reduce the sour taste. It is also remarkable that the three varieties taste differently sour.

Volatile acids

In Table 6 the volatile acid content of the three varieties are also given. The results show that white guava has the highest volatile acid content, followed by the pink guava, the Hawaiin hybrid having the lowest concentration. The above results could be due to higher sugar content in white guava, as the immediate precursors of organic acids are in general other organic acids or sugars (43). Two common volatile acids of fruits though present at low concentration are acetic acid and formic acid (43).

Very few reports are available on the volatile constituents of guava (42). It would therefore appear very interesting and desirable to investigate the guava flavour, since it seems to make this fruit and its products so appealing.

Presented in fig. 2 is the paper chromatogram of organic acids found in the guava juice and the organic acid standards used.

l - 7 represent the standard acids used; ascorbic acid, succinic acid, galacturonic acid, lactic acid, tartaric acid, malic acid, and citric acid, 8 represent the guava juice.

Fig. 2

Paper Chromatogram of Organic Acids

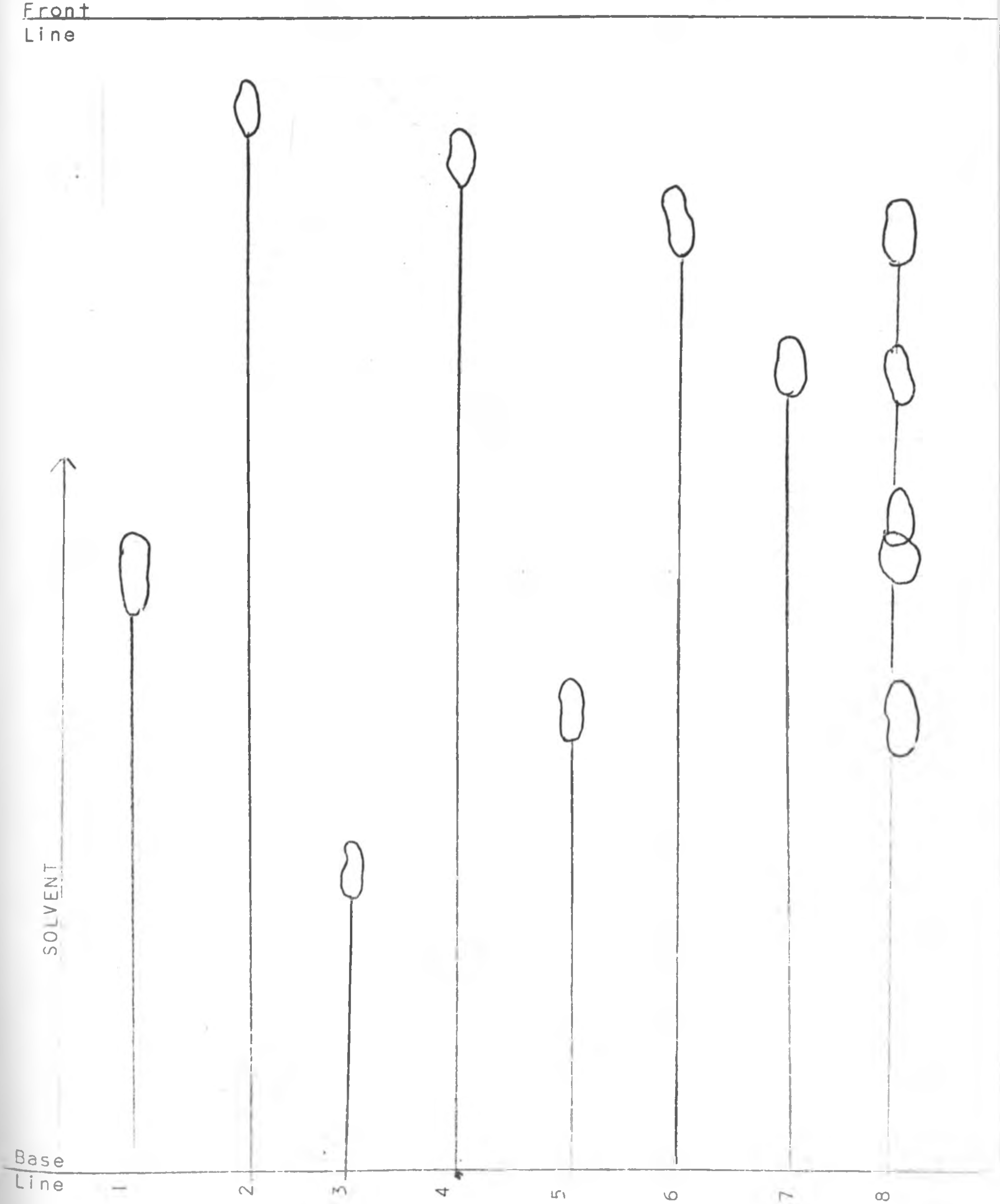


Table 7 shows the Rf values X 100 of the non-volatile organic acids. The organic acids were developed on B.B.I.F.W. using the descending technique.

Table 7: Rf values (X 100) of non-volatile organic acids (Paper Chromatogram)

Organic acids	Standard organic acids	Pink guava juice
Unknown 1	-	0 (does not move)
Galacturonic acid	20.3	19.7
Tartaric acid	40.9	36.7
Ascorbic acid	43.9	40.6
Unknown 2	-	45.3
Citric acid	55.7	54.3
Malic acid	63.4	60.6
Lactic acid	76	-
Succinic acid	80.0	-

During the preliminary experiments it has been found that paper chromatography was better for organic acid identification compared to the thin layer chromatography.

The following five acids were identified on both plate and paper, galacturonic acid, tartaric acid, ascorbic acid, citric acid and malic acid.

Two unknowns, 1 and 2, were also observed. Unknown 1 did not move and gave a blue colour with the location reagent. These are possibly basic compounds of guava juice, since basic compounds give blue colour with bromocresol green reagent (28). The blue spot appeared both on paper and thin layer chromatogram.

Presented in fig. 3, 4, and 5 are the standard curves for three non-volatile organic acids: citric, malic, and tartaric.

Fig. 5. STANDARD CURVE FOR MALIC ACID

Scale 20cm=1mg/ml

20cm=100sq. mm.

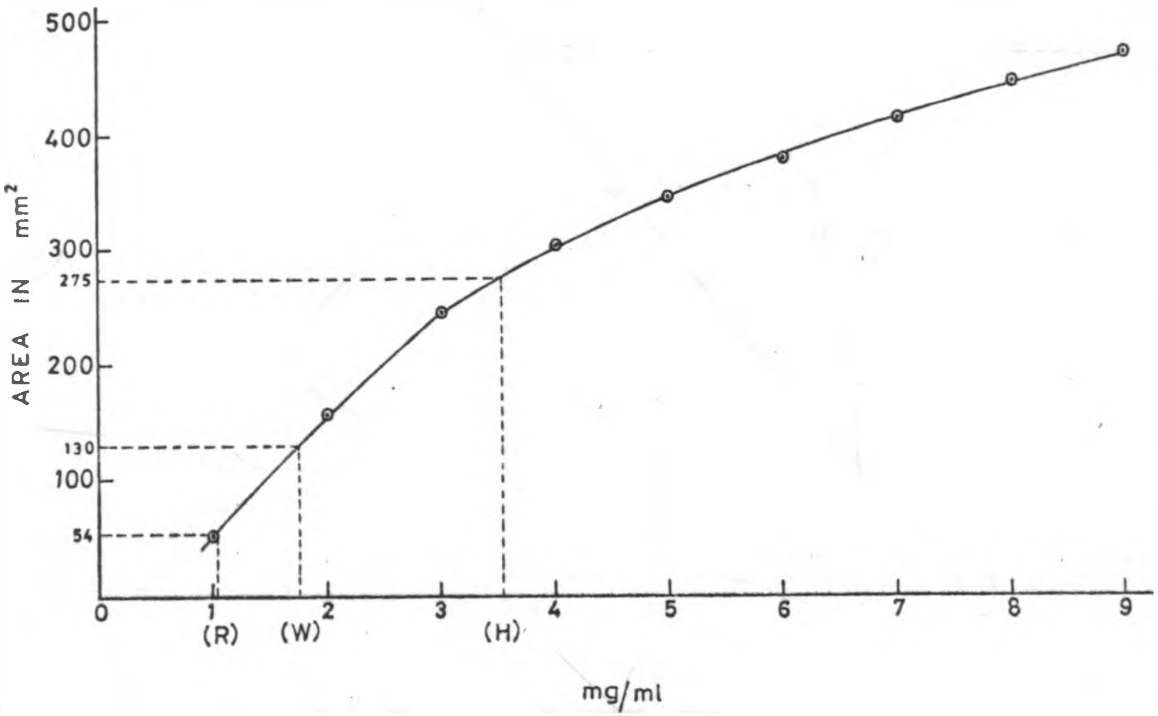


FIG. 4 STANDARD CURVE FOR TARTARIC ACID

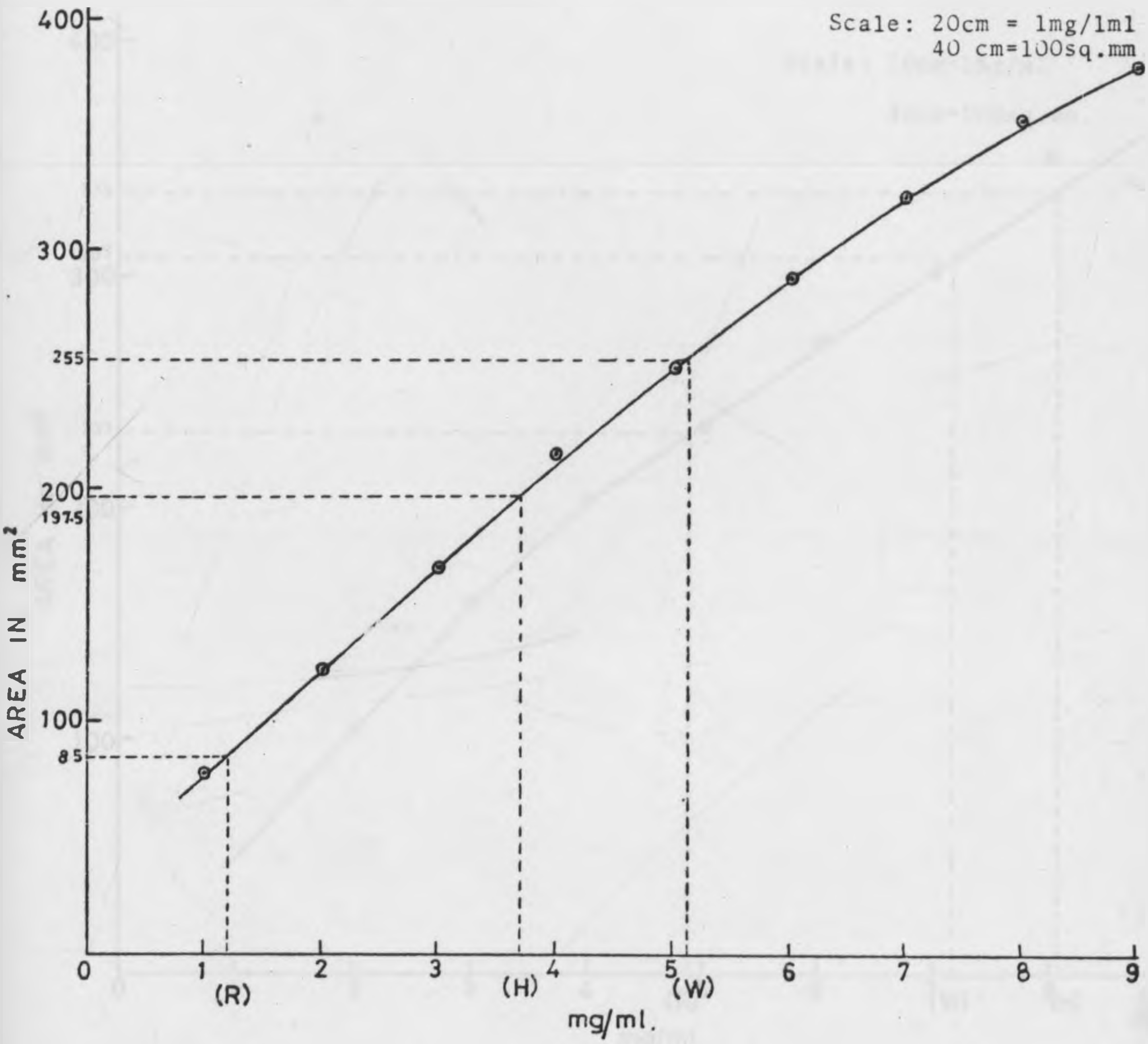
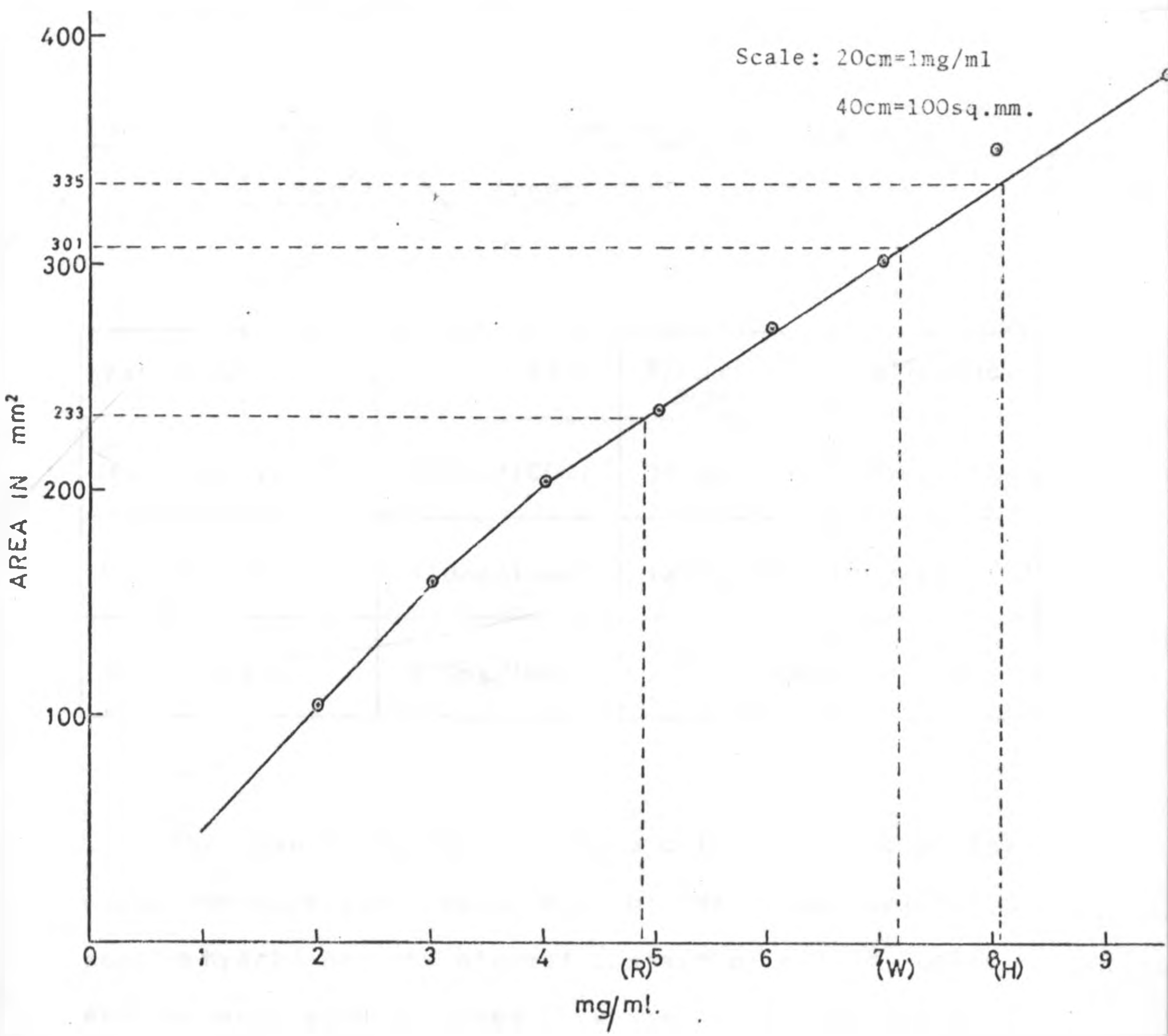


FIG. 6 STANDARD CURVE FOR CITRIC ACID



Presented in Table 8 are the concentration of three non-volatile organic acid in guava juice, obtained by using paper chromatography. The results are an average of four samples analyzed and are calculated in mg/100ml.

Table 8: Concentration of three major organic acids in guava juice (paper chromatography)

Varieties	Citric Acid	Tartaric Acid	Malic Acid
Hawaiin Hybrid	805mg/100ml	355mg/100ml	355mg/100ml
Pink Guava	440mg/100ml	120mg/100ml	105mg/100ml
White Guava	530mg/100ml	515mg/100ml	175/100ml

The results in Table 8 shows citric acid to be the major non-volatile organic acid of the three varieties, Hawaiin hybrid had the highest content of citric, malic and tartaric acid followed by white guava and lastly pink guava. This was not in agreement with the titratable acidity, where white guava had the lowest content. The above finding may be due to the high mineral content of white guava.

The following Table 9 shows the concentration of tartaric acid in guava juice obtained using a chemical method from International Federation of Fruit Juice Producers (18). The method applied is mostly used in analysing tartaric acid in grapes.

Table 9: Tartaric acid in guava

Varieties	Concentration mg/100ml
Hawaiin Hybrid	310mg/100ml
Pink Guava	201mg/100ml
White Guava	329mg/100ml

The above results are the average of four samples analysed per varieties.

The concentration of tartaric acid in the three varieties of guava examined and presented in Table 9 is more or less equal in the two guava varieties, Hawaiin hybrid and white guava. Tartaric acid is therefore not a major acid in guava. It is clear that citric and malic acids form the major acids of the guava varieties analysed in this work. Tartaric acid is reported to be uncommon in many fruits but is characteristic of grapes, but it has been found in raspberry and in avocado (43).

Santini (36) found tartaric acid in two varieties of guava Domnica Blanca Agri, and Puerto Rico Rosanda. The acid was also found present in small amounts by Chan et al. (8).

Tartaric acid content quantified by paper and chemical analysis was in agreement in the Hawaiin hybrid only. This could also be due to the fact that the chemical method of analysis used in this study is apllied to grapes.

Chan et al. (8) using gas liquid chromatography reported the contents of citric and malic acids to be 532mg/100gm and 469mg/100gm respectively in Beaumont guava, 541mg/100gm and 182mg/100gm in wild guava.

Comparing these figures with those in Table 8 it does show that concentration measured using paper chromatogram is possible to a high degree, provided precautions are taken.

Lactic acid

No lactic acid was found on analysis of Kenya guava juice. This is in agreement with Amerine and Cruess reported by Ryan (35), that lactic acid and succinic acid are products of alcoholic fermentation and not usually present in fresh fruit juices.

However, Chan et al. (8) reported the presence of lactic acid in guava. They identified the acid using thin layer chromatography. It is possible that the presence of over ripe guavas in that consignment may be responsible for the low content reported (12 - 25mg/100gm).

Table 10 shows the results of the ascorbic acid content in whole guava juice obtained by enzyme method and the ascorbic acid content of parts of the fruit; peel and pulp of unripe and ripe guava fruit. The analysis were done on fresh material.

Table 10. Ascorbic acid content of pink and white guava

	Pink guava	White guava
"Whole guava fruit treated with pectinex	39.5mg*	59.3mg*
Ripe guava peel	86.5mg**	173.0mg**
Ripe guava pulp	37.0mg**	37.0mg**
Unripe guava peel	123.5mg**	196.0mg**
Unripe guava pulp	74.0mg**	86.5mg**

"The juice used was made from fresh whole guava fruit.

*In mg/100ml of juice

**are in mg/100gm of fresh material.

It is possible that during the juice extraction, if not enough care is taken, some ascorbic acid may be lost. The juice of the white guava shows a higher content of ascorbic acid including the peel and pulp on both ripe and unripe guava. These results confirm those of El Faki and Saeed (12).

In comparing the ascorbic acid conient of the peel and the pulp the peel is found to have a higher content than the pulp, which is in agreement with other reports (12, 30). From these results it can be said that the peeling of guava would be detrimental to the nutritional value of the derived products.

The ascorbic acid is also found to be higher in unripe guava fruits than in ripe ones. This change of the ascorbic acid content is accompanied with changes of the total acidity of fruits.

The results in Table 10 indicate that the white guava has a higher ascorbic acid content than the pink guava. The results also confirm the high ascorbic acid content of guava which ranges well over 50mg/100gm of fresh whole fruit (8).

It should be kept in mind on comparison of different guava fruits that the varieties and the environmental conditions during growth affect the ascorbic acid content to a greater or lesser extent.

4.2. Sugars

Presented in Table II are the sugar content analysis of the three guava varieties.

Table II: Sugar Content of Guava Juice

Varieties	Total sugars	Reducing sugars	Sucrose	Soluble solids (^{o}Brix)
Hawaiin hybrid	3.60gm/100ml	2.16gm/100ml	1.36gm/100ml	9.1
Pink guava	2.80gm/100ml	1.97gm/100ml	0.78gm/100ml	10.1
White guava	4.38gm/100ml	2.69gm/i00ml	1.60gm/100ml	14

The above results are average of the analysis of four samples for each variety.

Table II shows the results for the analysis for total sugars, reducing sugars, sucrose and soluble solids content of the three varieties of Kenya guavas. The sucrose content was not directly analysed but derived from the total sugar content and reducing sugar content. The total sugar was measured after inversion as mentioned in 3.3.3. (33).

The results in Table II shows the white guava had a higher total sugar content followed by Hawaiin hybrid and pink guava. The total sugar content of many fruits lies in the region of 5 - 10% (43). Many determinations on total sugars and indeed many recent ones have been done by measuring total reducing sugars after inversion.

Sugar content as stated earlier in the literature review may vary considrably with the kind of soil and climatic conditions during the development of the fruit (43). In addition changes of sugar do occur between harvesting and full ripeness.

The reducing sugar results showed white guava had a higher content of reducing sugar compared to the other varieties.

The content of the reducing sugars is a measure of the total monsaccharide while sucrose a non-reducing sugar, is almost invariably the major disaccharide.

White guava is observed to have a higher content of soluble solids, which is due to the high content of sugar in white guava. From processors point of view, white guava would be considered too sweet for processing.

As mentioned earlier in the literature review, the determination of soluble solids is important to the commercial fruit processors in assessing guava fruits and the derived products, and also in product formulation.

The high content of sugar in fruits and their products are not usually recommended, as it may lead to browning reaction and lower the quality. Therefore, the determination of soluble solids in fruits and their products is a very important criterion in the commercial field.

Table 12 shows the Rf. values X100 of three sugars; sucrose, glucose and fructose identified using paper chromatography.

Table 12: Rf. (X100) of four sugars in guava (Paper Chromatogram)

Sugar	Standard	Guava
Sucrose	25	26
Glucose	30	31
Fructose	37	38
*Arabinose	38	-

*Arabinose reacted differently to the spraying reagent giving a pink spot while the other sugars showed brown spots. For this reason, the standard could not be identified to one of the spots resulting from the juice.

Fig. 6

Paper Chromatogram of Sugars in Guava Juice

In guava juice, glucose, fructose, and sucrose are definitely present. Though others (43) have identified arabinose its presence could not be confirmed, as it may be present in a very low concentration.

4.3. Mineral matter

Table 13 shows the results obtained in guava mineral content analysis. Individual minerals were not analyzed.

Table 13: Mineral content of three guava varieties

Varieties	Mineral Content
Hawaiin hybrid	0.62gm/100ml
Pink guava	0.38gm/100ml
White guava	0.61gm/100ml

The above results are averages of the analysis of four samples for each variety.

The mineral content of guava as in very many fruits is nutritionally valuable, since the minerals function as both building matter and regulatory substances in the body. The important minerals in guava are as follows: Calcium, Potassium, Sodium and Phosphate (3).

Calcium has two important functions in the body: to help build bones and teeth and to regulate certain body processes. Phosphate, together with organic materials forms structural units in every body cell and functions in almost every aspect of metabolism. Sodium being a major component of extra cellular fluid helps to maintain osmotic pressure. Like sodium, potassium helps to maintain osmotic pressure and the acid-base balance. The attributes of the mineral content for the body therefore warrants the analysis for the mineral content in guava

The mineral content of guavas lies between 0.38 - 0.62% as shown in Table 13, but the results are governed mostly by the type of environmental condition on which the fruit is grown and perhaps to a lesser extent on the climatic condition. In the literature, as stated earlier, the mineral content of guava lies between 0.2% - 1% (3, 19, 29).

5. CONCLUSION

Three varieties of guava which grow in Kenya were analysed for their chemical characteristics such as acids, sugars, and mineral content.

The pH was the same for the three varieties. Titratable acidity was high in Hawaiin hybrid and pink guava but lower in white guava. The results showed that Kenya guavas are relatively sour, and would need the addition of sugar in the juice for taste acceptance. Some workers have attempted to assess the stage of maturity in terms of acidity, although this factor is not usually constant. For this work, ripe guavas were used and therefore the acidity was not considered as a maturity criterion.

Guava is one of the fruits with a very nice appealing flavour, and since flavour is foundamentally the balance between acid/sugar ratio and volatile constituents, these characteristics deserved special attention.

Of the three varieties, the white guava had the highest volatile acid content. Very few reports are available on this topic. It would therefore appear desirable to investigate further the guava aroma.

Of the individual acids identified, citric acid has been confirmed to be the major non-volatile organic acid followed by malic acid, and tartaric acid. The Hawaiin hybrid had the highest content of the three acids, followed by the white guava and pink guava respectively. This was not in agreement with the titratable acidity where white guava had the lowest content. The above finding may be due to the high mineral content of white guava and to the possible salt formation between acids and cations. Lactic acid which has been reported by other workers was not present.

An important attribute of guava is the high content of ascorbic acid. Guava will become more and more important as the public learns to appreciate the high value of guava products in supplying this essential vitamin. From the three varieties, white guava would be recommended for processing due to the highest vitamin C content.

Ascorbic acid was found in higher concentration in the peel than in the pulp. It is therefore advisable not to remove the peel during processing, and at the same time eliminate the problem of waste.

The sugar content of guava was also of special interest. White guava had the highest content of total and reducing sugars. White guava would therefore be considered as acceptable to the taste when consumed fresh. On the other hand, they are not acceptable for processing as the high sugar content may easily lead to the browning of guava products.

The mineral content of guava is important in that the main minerals are: potassium, phosphate, calcium, sodium, which are essential minerals for the body requirements.

The study of organic acids, sugars, and mineral content will remain of importance as they have an important function in the plant metabolism, and also contribute important properties to the guava products, being mostly prominent in flavour, processing, preservation and nutritional values.

Since guava grow well on land considered marginal for other crops, there are many areas in Kenya where it would be feasible and profitable to grow the fruit as cultivated orchard crop. Of the three guava varieties it is recommended to cultivate only pink and Hawaiin hybrid for processing purposes.

From the above results, it can be seen that, as the popularity of guava fruit expands here and abroad, it becomes increasingly important to have a comprehensive knowledge of the guava chemistry.

6. SUMMARY

Three varieties Patnangola, Marherber and Hawaiin hybrid of guavas grown in Kenya were used for analysis.

The enzymatic method of juice preparation was preferred as it was relatively cheaper and quicker. The yield was not of interest in the juice preparation, and therefore was not calculated.

The acidity expressed as pH for the guava juice was 3.6. The highest content of titratable acidity of 1.49% was of the juice obtained from Hawaiin hybrid, followed by the juice of the pink guava and the white guava.

White guava had the highest volatile acid content of 60 mg/100 ml, followed by pink guava with 34 mg/100 ml, and lastly the Hawaiin hybrid with 24 mg/100 ml. Since very few reports are available on the volatile compounds of guava, it would be interesting and desirable to investigate the guava flavour.

Ascorbic acid varied from 120 mg/100 ml - 260 mg/100 ml in the fruit. White guava had the highest ascorbic acid content. Comparison between peel and pulp showed that the peel had a higher ascorbic acid content than the pulp. On comparing unripe and ripe guava, the results showed both the unripe peel and pulp to have a higher ascorbic acid content than the ripe peel and pulp.

Although other workers have reported the presence of lactic acid in guava, the acid was not found to be present in Kenya guavas.

Tartaric acid was present in appreciable amount, white guava juice contained 329 mg/100 ml, Hawaiin hybrid juice 310 mg/100 ml and pink guava contained 201 mg/100 ml. Tartaric acid is considered characteristic of grapes, and is only present in small quantities in other fruits.

Citric acid was confirmed to be the major non-volatile organic acid and present in the highest quantity. The amount varied between 805 mg/100 ml - 440 mg/100 ml in the three varieties analysed.

Calculation of the total sugar content, showed white guava juice had 4.38 gm/100 ml after inversion. The pink guava juice 2.80 gm/100 ml and the Hawaiin hybrid 3.60 gm/100 ml.

The reducing sugar content of guava juice samples showed that white guava contained 2.69 gm/100 ml, pink guava had 1.97 gm/100 ml and Hawaiin hybrid 2.16 gm/100 ml. White guava had the highest reducing sugar and total sugar content.

Sucrose content calculated from the total sugar contributed 33 to 42% of the total sugar in the three varieties analysed.

The soluble solids or sugars measured in ^{o}Brix were between 9 - 14 ^{o}Brix for the guava varieties analysed.

The mineral content averaged between 0.38 gm/100 ml to 0.6 gm/100 ml. The values depend more or less on the type of soil and the climatic condition to which the plant has been exposed.

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