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Biochemical composition of Soursop fruit, *Annona muricata* L., as affected by two harvest seasons

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ABSTRACT

Soursop, *Annona muricata* L., is a crop with promising economic value. The effects of season of harvesting and state of hardness on biochemical composition of the fruit juice were investigated. Analysis of variance of mean determinants of treatments, season versus hardness, was done and means separated by the LSD, p< .01. Value of pH for class 1 (hard-but-ripe) and class 11 (ripe-and-soft) were 3.6 and 4.6, respectively. High significance occurred for sugar content, 17.0 19.0% and 20.0 23.0% for wet and dry seasons harvests, respectively, identified as glucose, fructose and maltose. The organic acids were citric and malic. Titratable acidity, as citric acid, also significantly different, were 156.85 438.40mg/100ml and 297.60 842.24mg/100ml. Similar significance was reflected for other parameters including volatile acidity, as acetic acid, 6.0 39.0,g/100ml and 168.0 108.0mg/100ml, fixed acidity, 150.80 399.63mg/100ml and 129.6 735.53mg/100ml, ascorbic acid, 108.75 - 196.13mg/100ml and 166.21 204.01mg/100ml, respectively for wet season and dry season harvests. Percent protein was 1.19-1.71% and 0.50-1.40% for the wet and dry seasons, respectively. Soft fruits had alcohol content of 1.2% and 0.9% for wet and dry season harvests, respectively. Seasonal changes, rather than storage conditions, were thought to have influenced parameters determined.

Key words: Chemical composition, dry season harvest, hardness, seasonal changes, soursop juice, Wet season harvest.

INTRODUCTION

Soursop fruit, *Annona muricata* L., a native of tropical America and West Indies (Dalziel, 1948; Harrison *et al.*, 1969; ANON, 1975) hardly grows in temperate countries (Janick *et al.*, 1969; Purseglove, 1974). It was introduced to China, Australia and Africa (ANON, 1975) and thrives in the rain forest zones of West Africa (Glendhill, 1972; Adams, 1972). Observation showed that it grows throughout Southern Nigeria. The fruit, which has a pleasant aroma, is taken as dessert or made into a refreshing beverage (Dalziel, 1948; Sanchez - Nieva *et al.*,1970; ANON, 1975).

It can be processed without losing its aromatic flavor (Sanchez - Nieva *et al.*, 1970; ANON, 1975). Benero *et al.*,(1971) found that lye peeling of the fruit discolored pulp and impaired juice quality, but handpeeling proved adequate. Pulping was done with a 0.060- inch screen and a finisher with a 0.020-inch screen, which had higher extraction performance (Sanchez-Nieva *et al.*, 1970). Frozen pulp without addition of sugar could be used in the manufacture of nectar, soft drinks, ice cream and similar products and is served as nectar base when sugar is added.

Addition of ascorbic acid improved the puree quality. Benero *et al.*, (1974) prepared soft drinks using soursop, 15^0 Brix and a blend of soursop tamarind, 15^0 and 17^0 Brix, respectively and sensory evaluation showed that they were acceptable for one year and ten months, respectively.

Sanchez Nieva *et al.*, (1970) found that soursop pulp contained total solids, 16.6 - 18.6%; total sugar (as invert sugar), 11.6 - 12.5%; reducing sugars, 9.5 - 11.7%; acidity (as anhydrous citric acid), 0.89 0 - 096%; soluble solid, 15.8 - 17.4%; and pH 3.61 - 3.65. However, Chan and Lee (1975), using a gas chromatograph with flame ionization detector reported a lower total value for sugars (10.58%) detected as glucose, fructose and sucrose. In one study, Enweani *et al.*, (2004) reported that total carbohydrate was 12.52g/l of juice content. The study referred showed quality differences between ripe and unripe fruits of soursop and sweetsop, respectively. However, it does not appear that a study showing the effect of seasonal changes on quality parameters of the soursop juice has been conducted. This paper is on the biochemical analysis of soursop fruit harvested at two different seasons of the year.

MATERIALS AND METHODS

Collection of samples

Fruits were collected from Scot's farm, Ubiaja in Edo State, Nigeria. Selective plucking was done from treetops and fruits were immediately separated into two categories: ripe-andsoft and, hardbutripe fruits known by the yellowish taint of the green oblong-shaped berry (Fig.1). All collections were done within one week in order to avoid physiological variation. The dry season collection was made in January, while the wet season collection was done in June of the same year.

Fig. 1: A ripe soursop (*Annona muricata* L.) fruit.

Labeling and storage of samples, juice preparation

Wet and dry season harvests (S and Z) were labeled as SS_1 or SS_2 and Z_1 or Z_2 for hard-but-ripe or ripe-and-soft fruit samples. Dry season samples were stored in a deep - freezer at 18°C for 6 months. The fruits were peeled, cored and seeds separated from pulp (Fig. 2); pooled puree thus obtained for each sample was analyzed. The pH of extracts were taken at 20°C using a pH meter, model 7020 (Electronic Industries Ltd) equiped with a glass electrode. Except otherwise stated, all determinations were done in triplicate.

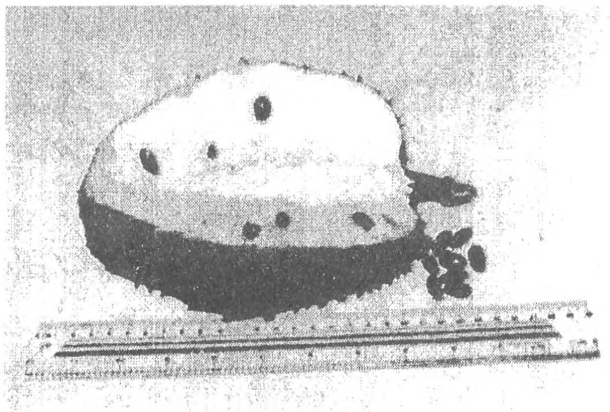

Fig. 2 : A sectioned fruit of soursop showing core, pulp and seeds. C, core; P, pulp: S, seeds.

Determination of sugar content

Standard D-glucose solutions were prepared and developed using the anthrone method (AOAC, 1970). Optical density (O.D.) of the solutions was determined at 625nm (Sharma *et al.*, 1984). A standard curve was constructed by plotting O.D. readings against concentration gradient. A 0.05ml of each juice sample was similarly treated. The concentration of sugar in the juice was found by extrapolating from the standard curve.

Determination of titratable acidity

A 20ml of centrifuged (at 3000g for 5min) soursop extract was transferred to a 250ml flask and 10 drops of phenolphthalein were added. A white tile was placed under the flask and sample was titrated against 0.1N NaOH from a burette until a definite pink end point was attained. Titratable acidity (as citric acid) per 100ml juice was obtained according to AOAC (1970).

Determination of volatile and fixed acidity

Volatile and fixed acidity of fruit extracts were determined by the standard methods (AOAC, 1970).

Determination of alcohol content by volume

This was done according to the description by AOAC (1970).

Determination of ascorbic acid content

Soursop extract was added to 200ml freshly prepared 0.6%TCA + 0.005M Na_2 EDTA in a 500ml beaker and mixed thoroughly. A 20ml of this solution was transferred to 100ml volumetric flask and made up to mark with 0.3% TCA + 0.0025M Na_2EDTA and mixed thoroughly. This was filtered through fluted Whatman No. 42 filter paper into a 150ml beaker to remove suspended solids. The filtrate was subjected to further analysis following the description in methods of vitamin assays (A.O.V.C., 1966).

Determination of protein content

Digestion of sample was as described by AOAC (1970) but with slight changes as in the modification of IITA (1979). A 5ml sample was measured into 500ml round bottom flask into which 5ml conc. H_2O_2 was added in a fume chamber and mixed. The mixture was placed on a heating rack with temperature raised in steps until 50°C when it boiled. Further measures of H_2O_2 were added to ensure complete digestion. A blank with distilled water was similarly treated.

Distillation of ammoniacal nitrogen was done following the method described in AOAC (1970). Crude protein was calculated thus,

$$\text{nitrogen} = \frac{\text{sample titre-blank titre} \times 0.56}{\text{Weight of sample}}$$

$$\%\,\text{protein} = \frac{\text{sample titre- blank titre} \times 0.56 \times 6.25}{\text{Weight of sample}}$$

Chromatographic analysis of sugar

This was done using a one-dimensional descending paper chromatography. Drops of samples and reference standards were spotted 3cm apart on Whatman No. 1 filter paper (40 x 45cm) with 0.1cm glass tube and quickly dried under a stream of hot air. The reference sugar solution contained 1% of each of arabinose, fructose, glucose, galactose, glycerol, lactose, maltose, raffinose, sucrose and xylose in distilled water. The chromatograph was equilibrated overnight in a chromatank (82.5 x 23 x 82.5cm internal dimensions, Shandon Sci. Co., London) with 23ml of solvent system, n-butanol-acetic acid-water (4:1:1). Subsequently, the chromatogram was run for 18h, dried in air and developed in silver nitrate solution according to the method of Trevelyan *et al.*, (1950). Dark-brown spots against a white background showed the presence of sugars. The strip was washed in 5% sodium thiosulphate and thereafter in running water for 1h. Sugars in samples were identified by comparing Rf ' values of spots with that of reference sugars run simultaneously, where Rf ' was given by the ratio, distance traveled by substance /distance traveled by solvent front.

Chromatographic analysis of organic acids

The method of Kunkee (1968) was employed. The solvent system was n-butanol-distilled water-formic acid- bromocresol green (100: 100: 10.7:15). The solvent mixture was shaken for 20min in a separation funnel and allowed to settle. The aqueous layer was let out and the organic layer used. Spotting, equilibration and development of chromatogram were done as described using acetic, fumaric, lactic, citric, glutaric, malic, oxalic, succinic, and tartaric acids as reference standards. The chromatogram was run overnight, and then air-dried at room temperature. Spots appeared with yellow color against a blue-green background. Identification was done as described.

RESULTS

The Rf' values of sugar and organic acids in soursop juice, measured against their standards in each case, were shown in Tables 1 and 2.

Table 1: ***Rf 'values of spots of standard sugars and soursop juice extracts.***

Sugar	Rf(x100) in n butanol	- acetate -	water
Arabinose	26.68		
Fructose	26.20[a]		
Glucose	12.99[a]		
Galactose	18.02		
Glycerol	53.13		
Lactose	6.49		
Maltose	7.93[a]		
Raffinose	3.85		
Sucrose	13.76		
Xylose	30.53		
S1[b]	7.29	12.60	23.95
S2	7.28	12.81	26.00
Z1	7.28	12.50	26.10
Z2	7.30	12.70	25.90

[a] Sugar Rf' values corresponding with fruit sample Rf's.
[b] S1, S2, Z1, Z2 are wet and dry season soursop juice samples.

Both classes of fruit hardness had a range of pH 3.60-4.40. Means of parameters determined for juice were shown in Table 3. There was significant difference, $p < .01$, between the levels of sugar determined for class 1 and 11 hardness, with higher values for the softened fruits. This means that as the fruit softened higher levels of sugar accumulated in the juice. This trend was also reflected in juice contents of ascorbic acid, titratable acidity and fixed acidity. The sugar content was 17.03 -23.00%. This was chromatographically detected as glucose, fructose and maltose (Fig.3).Chromatographic analysis of organic acids showed the presence of citric and malic acids (Fig. 4). The Rf' values of sample sugars and organic acids were lower than those of standards. Total titratable acidity was 156.85 - 842.24mg/100 1. Volatile acidity, measured as acetic acid had a range from 6.0 168.00mg/100ml, while fixed acidity as expected, was much higher (129.67 - 735.53mg/100ml). The ascorbic acid content was high, 108.75 -204.01mg/100ml, but the protein content did not exceed 1.71%. Specific gravity bottle determination of alcoholic content was done for ripe-and-soft fruits only. This was found to be 0.9 and 1.2% for dry season and wet season harvests, respectively.

Table 2: ***Rf' values of spots of organic acid standards and soursop juice.***

Acid	Rf (x100) in n-butanol-	formate-water
Acetic acid	90.60	
Glutaric acid	92.60	
Citric acid	63.06[a]	
Fumaric acid	95.67	
Malic acid	70.00[a]	
Lactic acid	86.77	
Glyoxillic acid	56.40	
Oxalic acid	37.50	
Succinic acid	86.56	
S1[b]	62.50	70.62
S2	62.50	69.70
Z1	62.50	70.00
Z2	6.250	69.88

[a] Acid Rf' values corresponding with sample Rf's.
[b] S1, S2, Z1 and Z2 are wet and dry season soursop juice samples.

Table 3: Chemical attributes assessed in juice of soursop fruit harvested in two different seasons

Fruit properties		Sugar %		Ascorbic acid mg/100ml		Protein %		Titratable acidity mg/100ml		Volatile acidity mg/100ml		Fixed acidity mg/100ml		Alcohol content %	
Hardness	Class	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season
	1	17.0	20.0	108.3	116.16	1.16	0.5	156.85	297.57	6.0	168.03	150.85	129.54	ND	ND
	2	19.3	23.0	196.45	202.89	1.71	1.4033	438.40	842.24	39.01	108.00	399.39	734.24	1.21	0.90
		P<0.01 Lsd: Season, 0.1195; Hardness, 0.1195		P<0.01 Lsd: Season, 1.109; Hardness, 1.109		P<0.01 Lsd: Season, 0.0261; Hardness, 0.0261		P<0.01 Lsd: Season, 0.3391; Hardness, 0.3391		P<0.01 Lsd: Season, 0.1501; Hardness, 0.1501		P<0.01 Lsd: Season, 0.2693; Hardness, 0.2693			

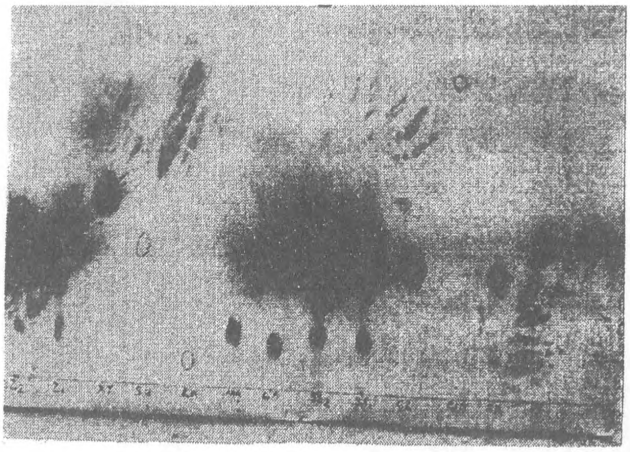

Fig. 3

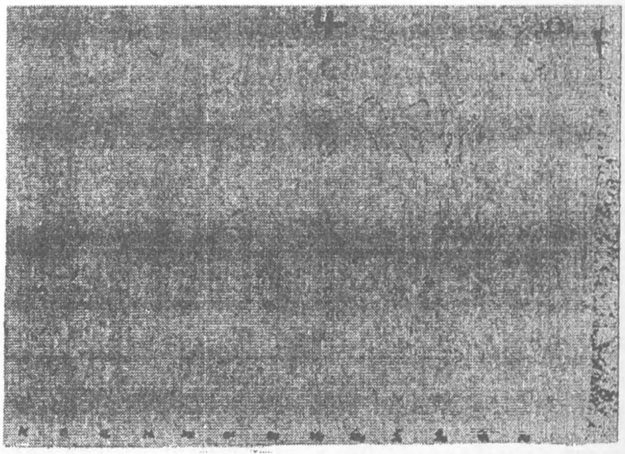

Fig. 4

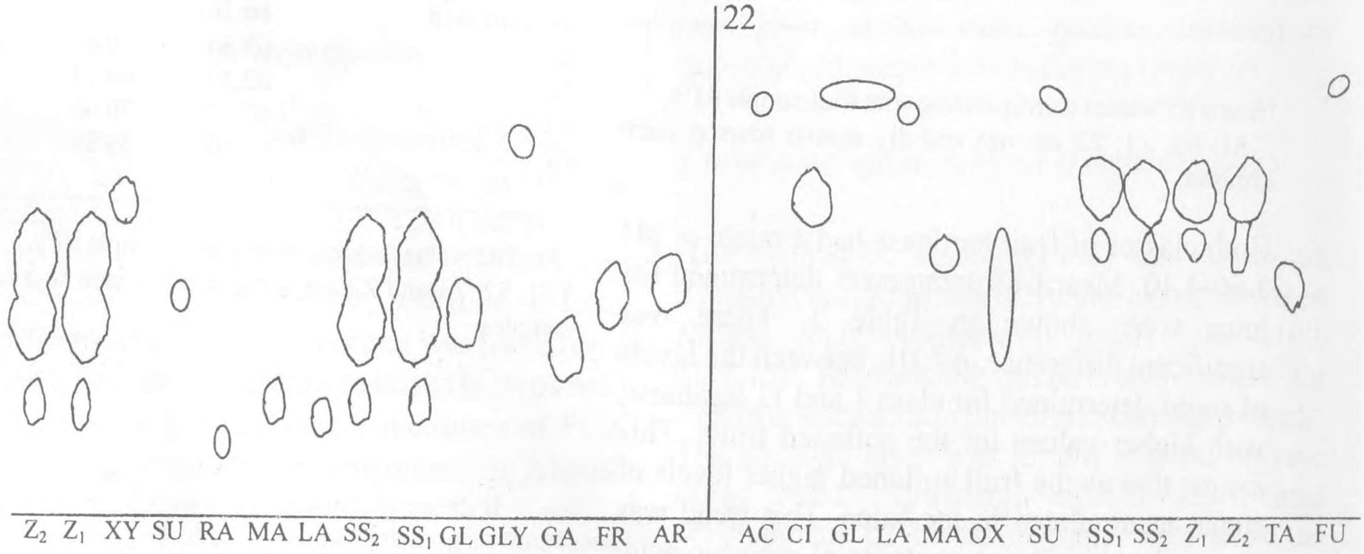

Fig.3: Chromatographic detection of sugars in soursop fruit extract. The solvent system was – butanol: acetic acid: water (4:1:1) Symbols: AR,arabinose; FR, fructose; GA, galactose; GL,glucose;GLY, glycerol; LA, lactose; MA, maltose;RA, raffinose; SU, sucrose; XY, xylose;SS_1, freshly harvested matured and ripe fruits; SS_2, freshly harvested ripe and soft fruit; Z_1, matured and ripe fruit stored for 6 months;Z_2 ripe and soft fruit stored for 6 months.

Fig. 4: Chromatographic detection of organic acids in soursop fruit extract. The solvent system was n-butanol: distilled water:formic acid: bromocresol green (100:100:10.7:15). AC, acetic acid; CI, citric acid; FU, fumaric acid; GI, glutaric acid;MA, malicacid; OX, oxalic acid; SU, succinic acid; YA, Tartaric acid;SS_1,SS_2,Z_1 andZ_2as in fig.3.

Dry season samples had higher values of percent sugar, titratable acidity, volatile acidity, pH and ascorbic acid in comparison with the wet season harvests. But this was not so for fixed acidity and percent protein where the wet season lot had higher values.

Table 3 showed that softened fruits in either seasons contained high percent sugar. The sugar content of juice increased from 17.00% to19.30% for the wet season harvest, and from 20% to 23% for the dry season harvest. Similar increase was also reflected in the total titratable acidity, where the wet season lot increased from 156.85mg/100ml to 438. 4 mg/100ml. The dry season lot also increased from 297.60mg/100ml to 842.24mg/100ml. But while there was increase in volatile acidity for the wet season crop, 6.0 to 39.0 mg/100ml, the dry season harvest showed a decrease (168 to 108mg/100ml), as the fruit softened.

DISCUSSION

In this study, ripe-andsoft fruits were analyzed along side hard but ripe fruits. Differences in sugar content may be due to hydrolytic enzyme activities that accompany ripening and softening, which result from solubilization of pectic substances associated with the middle lamellae (Leopold and Kriederman, 1975), when fats and starch also yield sugars (Beevers, 1961). Similarly, higher values for titratable acidity, volatile acidity, fixed acidity, ascorbic acid and protein in softened fruits may be attributed to effect of hydrolysis of more complex parent compounds. Hulme (1954) showed that protein synthesis accompanied the ripening of apple fruits.

Fruits stored at -18°C for 6months presented higher values, $p < .01$, for sugar, titratable acidity, volatile acidity, fixed acidity and ascorbic acid. Leopold and Kriederman (1975) noted that plant tissues stored at temperatures below 10°C increase in sugar concentration. It is difficult to attribute the increases in values in the present study to the low storage temperature and, or period, since a portion of the dry season harvest was not analyzed at the time of harvest. However, Sanchez-Nieva *et al.* (1970) showed that unsweetened soursop puree with or without heat- treatment and stored at -10°F for 223-225 days or 396-419 days did not show significant change in total solids, pH and color. In our study, it is tempting not to attribute the differences observed to the influence of season since environmental conditions differed greatly between the two harvesting periods.

The evergreen soursop plant which flowers and fruits almost continuously throughout the year (ANON, 1975), has a tap root system for meeting physiological water requirement, in spite of low relative humidity and high atmospheric temperature that occur in the dry season. Results in this study showed that the dry season harvests had higher values for sugar, titratable acidity, fixed acidity, and ascorbic acid, in comparison with fruits harvested in the wet season, when humidity was relatively high. Seasonal differences may also account for the lower protein value recorded for the dry season harvest.Volumetric measurements showed the presence of alcohol in ripe and soft fruits harvested in both season, with a higher value for the wet season crop. The difference in pH of softened fruits between the two seasons is insignificant to the extent that it is within the acidic range.

Malic and citric acids were detected in the dry- and wet- season crops. Citric acid is a product of the photosynthetic carbon reduction cycle in C_3 plants (Ting and Osmond, 1973), while malic acid occurs as an intermediate product in C_4 plants, but it is quickly metabolized to other products (Edwards and Haber, 1981). In CAM plants, CO_2 is fixed in malic acid in the dark (Beevers *et al.*, 1966), and eventually released from storage in light for the photosynthetic carbon reduction process (Edwards and Haber, 1981; Leopold and Kriederman, 1975). Since malic and citric acids were the only organic acids detected in soursop juice, one may speculate on the occurrence of CAM process in soursop. It is known that crassulacean acid metabolism is a photosynthetic and biochemical adaptation of CO_2 utilization to water stress (Osmond and Holtum, 1981), a condition that possibly prevails for the crop in the dry season. If this is assumed, then the higher sugar, titratable acidity, fixed acidity and ascorbic acid values detected may not be unexpected. The lower value for volatile acidity in dry season softened fruit cannot be accounted for presently.

The total percent sugar (19-23%) in ripe-and-soft soursop fruits is higher than that presented by other workers, 12% (ANON, 1975); 10.58% (Chan and Lee, 1975); 11.6-12.5% (Sanchez-Nieva *et al.*, 1970). The value determined in this study compares favorably with that for grape (*Vitis* sp.) must (18-25%), the conventional wine Making fruit (Amerine, 1964; Okafor, 1987). Therefore, soursop may serve for wine production.

Sugars previously reported in soursop fruit include invert sugar (Sanchez-Nieva *et al.*, 1970), glucose, fructose and sucrose (ANON, 1975; Chan and Lee, 1975). A new discovery in this study is the detection of maltose, disaccharide and reducing sugar, which occurs naturally in plants (Conn and Stumpf, 1976). This appears to be the first report on the detection of maltose in soursop fruit juice.

Several papers (Sanchez-Nieva *et al.*, 1970; Benero *et al.*, 1974; ANON, 1975) have reported on the use of soursop puree for the manufacture of nectar, ice cream, soft drinks and other similar products. The high concentration of sugar makes the juice suitable for alcohol fermentation, without further amelioration, which is done when the sugar content is low (Jarczyk and Wzorek, 1977). It's acid pH and aroma (ANON, 1975), may contribute to a high quality product.

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