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Nutritional, Organoleptic and Phytochemical Properties of Soursop (*Annona muricata*) Pulp and Juice after Postharvest Ripening

Bernard Tiencheu[1*], Agbor Claudia Egbe[1], Aduni Ufuan Achidi[1], Noel Tenyang[2], Eurydice Flore Tiepma Ngongang[1], Fabrice Tonfack Djikeng[3] and Bertrand Tatsinkou Fossi[4]

[1]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P. O. Box 63, Buea, Cameroon.
[2]Department of Biological Science, Faculty of Science, University of Maroua, P.O. Box 814, Maroua, Cameroon.
[3]School of Agriculture and Natural Resources, Catholic University Institute of Buea, P.O Box 563, Buea, Cameroon.
[4]Department of Microbiology and Parasitology, Faculty of Science, University of Buea, P. O. Box 63, Buea, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Authors ACE and BT designed the study, performed the statistical analysis and conducted the work, wrote the protocol, and wrote the first draft of the manuscript. Authors EFTN, FTD and BTF managed the analyses of the study. Author AUA vizualised, managed the literature searches and supervised. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Annona comprises many species but four are known as bearers of edible fruits, namely, *A. reticulata*, *A. squamosal*, *A. cherimola* and *A. muricata.* Soursop is not quite exploited in Cameroon. This work was aimed at determining the nutritional and phytochemical properties of soursop pulp and formulated juices.

**Corresponding author: Email: tiencheu.bernard@ubuea.cm, bernardtiencheu@yahoo.fr;*

Methodology: The proximate composition, mineral and phytochemical contents of the pulp and formulated juice with food additives were determined using standard methods. The sensory evaluation of the juice was conducted using a 9- point hedonic scale.
Results: Overall acceptability of the juices ranged from 3.87- 6.38. The qualitative phytochemical screening of the juices showed major secondary metabolites (alkaloids, saponins, tannins except steroids and glycosides). Quantitatively, total phenolic content ranged from 0.26-0.89mg GAE/ mL. Proximate composition of the pulp and juice also varied showing; protein (0.58-7.45%), lipid (0.10-0.74%), fibre (1.26-24.23%), ash (1.29 to 2.22%), carbohydrate (8.63-21.0%) and moisture (45.0-88.23%).The minerals in the pulp and juice were; K (7.70-10.06mg/g), Fe (0.19-0.42mg/g), Ca (0.96-2.16mg/g), Mg (0.19-0.68mg/g), Zn (0.04-0.08mg/g), P (0.05-1.23) and Na (0.73-0.81mg/g). Inclusion of additives to the juice generally increased the acceptability of consumers.
Conclusion: It can be concluded that soursop pulp and juice contain appreciable amounts of nutrients and phytochemicals which can be exploited to improve nutrition and health. This may contribute to an increase in itsconsumption, a reduction in postharvest losses and an increase in domestication of soursop plant.

Keywords: Postharvest; nutrient; food additive; juice; soursop sensory evaluation; Annona muricata.

ABBREVIATIONS

P3 : Pulps for 3 days postharvest ripening soursop
P5 : Pulps for 5 days postharvest ripening soursop
EP3 : Extract from Pulps day 3 ripening soursop
EP5 : Extract from Pulps day 5 ripening soursop
EP3F1 : Extract from Pulps day 3 ripening soursop Formula 1
EP3F2 : Extract from Pulps day 3 ripening soursop Formula 2
EP3F3 : Extract from Pulps day 3 ripening soursop Formula 3
EP3F4 : Extract from Pulps day 3 ripening soursop Formula 4
EP5F1 : Extract from Pulps day 5 ripening soursop Formula 1
EP5F2 : Extract from Pulps day 5 ripening soursop Formula 2
EP5F3 : Extract from Pulps day 5 ripening soursop Formula 2
EP5F4 : Extract from Pulps day 5 ripening soursop Formula 4

1. INTRODUCTION

The name annona derives from the Latin “annual harvest” [1]. The genus presents numerous unifying characteristics, especially relating to plant height, root system, bark, stem, floral biology, pollination, fruit set and fruit type [2-4]. There are important variations among annona seedlings in the same species, affecting not only the mature foliage and productivity of the plants, but also the fruits size, form, colour, quality and number of seeds in the fruits. These variations are often pronounced enough to have resulted in several botanical names for the same species. In general, the annonas are shrubs or small trees, whose height varies from 5 to 11m depending on several factors, such as species, climate, soil and crop management. They are erect or somewhat spreading in habit, with grey brown bark, often rough and corrugated [4]. All portions of the soursop tree similar to other Annona species, including *A. squamosa* and *A. reticulata* are extensively used as traditional medicines against an array of human ailments and diseases, especially cancer and parasitic infections. The fruit is used as natural medicine for arthritic pain, arthritis, diarrhea, dysentery, fever, malaria, parasites, rheumatism, skin rashes and worms, and it is also eaten to elevate a mother’s milk after childbirth [5]. The soursop fruit is a compound fruit and covered with reticulated, leathery but appearing tender, inedible bitter skin from which protrudes few or many stubby or more elongated and curved soft, pliable “spines”. The skin is dark-green in the immature fruit, becoming slightly yellowish-green before the mature fruit is soft to the touch. In aroma, the pulp is somewhat pineapple-like, but its musky, sub acid to acid flavor is unique. It is indigenous to most of the warmest tropical areas in Africa, South and North America including Amazon, *A. muricata* has become naturalized in many countries, and now has a wide distribution throughout tropical and subtropical parts of the world [5].

Juices produced from tropical fruits have increasingly gained global importance due to their characteristic exotic aroma and colour. There are different types of tropical fruits readily available for the production of fruit juice. These include oranges, grapes, pineapple,

banana, guava and watermelon. Their uses depend on the type of drink or juice one intends to produce. The juice may be produced from single fruit or a combination of fruits of different choices. Fruits may also be processed into other fruit products such as beverages, wine, jellies and jam. There are fruits that are less well known or highly dispersed. Such fruits include lychee, babaco mamay and soursop [6]. The *Annona muricata* fruit makes an excellent drink or ice cream after straining. Several studies have described the medicinal purposes of *Annona muricata* and have outlined the social history of the plants. Soursop is the most versatile Annona fruit for industrial purpose because it does not oxidize easily and there is a large recovery of pulp from the fruit during processing [7]. Fruit juices are widely consumed in ever increasing quantities and very important soft drinks in the trade of most countries. Juices are healthy beverages because they contain some vitamins and minerals from the original fruit [8].

A wide diversity of compounds especially secondary metabolites, found in plants have been found to have anticancer, antibacterial, anti-inflammatory, antitumor, antiviral and many other activities [9,10]. Distinguished examples of these phytochemical compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides, stilbenes, tannins, nitrogen compounds (alkaloids, amines, betalains), terpenoids and some other endogenous metabolites [9-11]. Soursop may also be rich in these secondary metabolites. Hence, it is of absolute importance that optimum postharvest maturity is well defined to reduce postharvest losses and attain 'acceptable' eating quality after storage [12]; To date, there is no available postharvest method or technology capable of extending the shelf life of the soursop fruits to more than 4-9 days if the fruits are stored at a temperature between 15 and 20°C [13]. Maximizing the benefits from soursop requires increased shelf-life and product development by selecting the optimal storage time that leads to optimal ripening, optimal nutrients content and optimal taste. This can be achieved by formulating juice from it. Adequate knowledge of the nutritional and phytochemical properties of soursop pulp and juice will better bring out its potentials thus this work is aimed at determining the nutritional and phytochemical properties of soursop fruit juice and pulp after 3rd and 5th day postharverst storage (ripening).

2. MATERIAL AND METHODS

2.1 Sample Collection

Mature soursop fruits were purchased from Muea market, South West Region of Cameroon. The fruit's maturity was determined by its dark green skin with smooth numerous fleshy spines. The samples were transported to the Life Science Laboratory, University of Buea, for sample preparations and analyses.

2.2 Sample Preparation

Samples were kept to ripen for 1, 3, 5, 7 and 9 days prior to usage at ambient temperature (25 °C) and a relative humidity (RH) of 85–90%. Finally 0 to 2 day fruits batches were so hard to be used, and after day 5 the remaining fruits got bad and spoiled. Batches for 3rd and 5th day were retained for the continuation of the study. Prior to peeling, fully ripe soursop fruits were washed under running tap water and rinsed with chlorinated water to minimize the risk of contamination.

2.3 Extraction of Pulp Juice

Pulps were obtained from the mature fruits left to ripen for three days (3) and five (5) days respectively at room temperature. Ten soursop fruits weighing averagely 200g each for the 3rd and 5th day of ripening were weighed using a weighing balance. They were washed, peeled and deseeded to obtain the pulp which were labelled P3 and P5 respectively. A part of P3 and P5 were stored at -20°C for proximate and minerals analyses and the other part used for pulp concentrate. Fig. 1, shows a flow chart of the work. 400 mL of water was added to 1kg of each pulp (P3 and P5) and blended for 10 minutes using a blender, sieved and filtered through a muslin cloth to obtain a clear pulp concentrate [14]. The concentrates obtained respectively were labelled EP3 and EP5 and kept for proximate and mineral analyses as well as for juice formulation.

2.4 Preparation of Fruit Juice Additives (Coloured Water) and Juice Formulation

Juice was formulated from the pulp concentrate (EP3 and EP5) by mixing different volumes of concentrate (Table 1) with coloured sweetened and flavoured water which was prepared as follows: 0.1g of egg yellow colour (E102, E110)

was dissolved in 550mL of Supermont water. Two drops of concentrated green food colour (E102; 1.8%), E110; 0.4%), 3mL of pineapple flavour and 30g of vanilla sugar were added to give an intermediate yellow colour, flavour and taste. The coloured water was used to complete each juice formulation to 100mL except samples EP3F1 and EP5F1 which consists of the only pulp concentrate with no colour, sugar or flavour added, these were used as control. Table 1, shows the recipe for the various juice formulations.

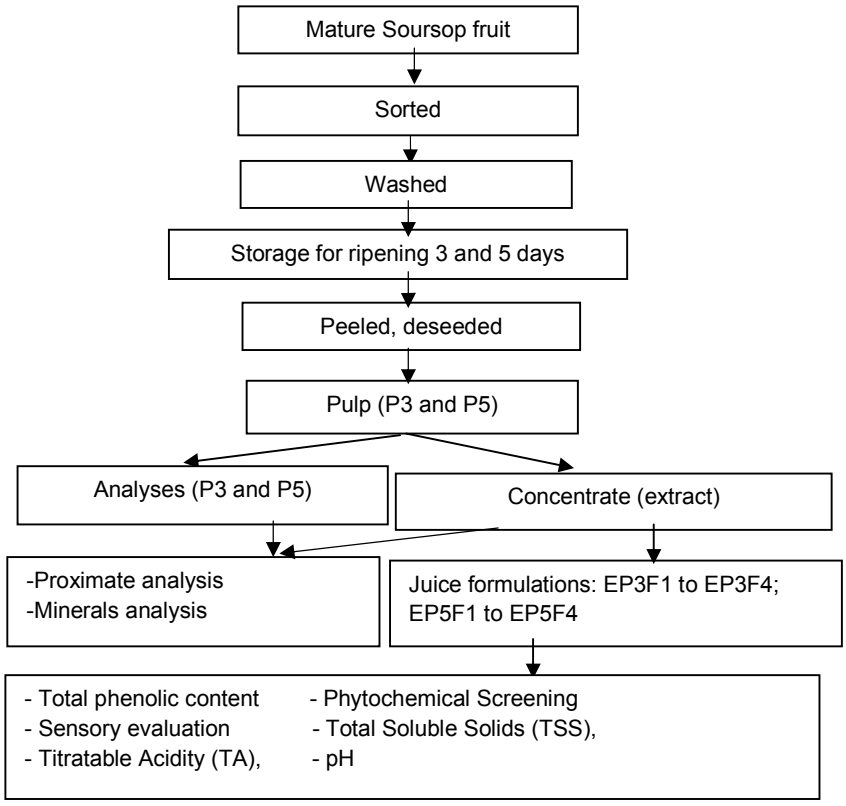

Fig. 1. Schematic presentation of the preparation of sample and flow chart of the work

P3: Pulp for 3rd day postharvest ripened soursop; P5: Pulp for 5th day postharvest ripened soursop; EP3: Concentrate from pulp of 3rd day ripened soursop; EP5: Concentrate from pulp of 5th day ripened soursop. EP3F1, EP3F2, EP3F3 and EP3F4: Four Juice formulations (1 to 4) from 3rd day ripening concentrate. EP5F1, EP5F2, EP5F3 and EP5F4: Four juice formulations (1 to 4) from 5th day 5 ripening concentrate

Table 1. Formulation of soursop juice using additives and extracts

Day (Ripening)	Juice formula code	Pulp concentrate (mL)	Coloured water (mL)	Total volume (mL)
3	EP3F1	100	0	100
	EP3F2	80	20	100
	EP3F3	60	40	100
	EP3F3	40	60	100
5	EP5F1	100	0	100
	EP5F2	80	20	100
	EP5F3	60	40	100
	EP5F3	40	60	100

EP3: Juice formulated from pulp concentrate of 3rd day ripened soursop; EP5: Juice concentrate from pulp extract of 5th day ripened soursop. F1 to F4: are they 4 juice formulations from each of the pulp extracts respectively with 100, 80, 60 and 40mL of extract

2.5 Proximate Analysis of Pulp and Pulp Concentrate

Proximate analysis of the samples (Pulps P3 and P5, concentrates EP3 and EP5) were carried out as follows: Moisture content was determined using the hot air method described by Association of Official Analytical Chemist (AOAC) [15]. Ash, protein, fat, and crude fiber were determined according to the AOAC method [15]. Carbohydrate content was determined by difference by subtracting the sum of percentage moisture, ash, protein, fat, and crude fiber was subtracted from 100. Percentage (%) carbohydrate = 100 – (% moisture + % ash + % protein + % fat + crude fiber)'. Na, P, Zn, Mg, Ca, Fe and K were analysed using the Association of Official Analytical method [16].

2.6 Analysis of Formulated Juices

The analysis of the 8 formulated juices (4 formulations with each concentrate) started with sensory and organoleptic analysis to select the best and highly acceptable juice. An effective acceptability test was applied to choose the two best juice. This selection was performed based on taste and colour acceptability (EP3F3 and EP5F3); EP3F1 and EP5F1 were maintained as a control (without additives).

2.6.1 Sensory evaluation

A 9-point hedonic scale was used to assess the colour, taste, flavour, texture and overall acceptability of the 8 formulated juices by 20 semi-trained panelists [17] who consisted of students, teachers and staff around the university campus and the best formulation selected for each ripening day. The taste panelists were asked to rate the different juices presented to them on a 9 point hedonic scale with the ratings of: 9 = like extremely; 8 = like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much and 1 = Dislike extremely. All the panelists tasted the different formula at 23°C. The means of the scores by the judges were analyzed for significant differences between their respective juice samples.

2.6.2 Determination of pH

Ten milliliters of a juice was dispensed into a beaker and the pH was determined with a previously standardized pH meter. The pH meter was calibrated using phosphate buffer of pH 4.0 and 7.0 [16].

2.6.3 Determination Total Titratable Acidity (TTA)

Standard method of Antony and Chandra [18] and Ferrati et al., [19] were used to measure the titratable acidity. Five grams of concentrated fruit juice was homogenized in distilled water (20mL) and filtered through Whatman No. 1 filter paper. Phenolphthalein was added to 20mL of the filtrate as indicator and titrated against 0.05M NaOH. Titratable acidity was calculated using the equation:

$$TA = (M \times NaOH \times 0.09 \times 100)/V$$

Where: TA: Titratable acidity; MNaOH: Molarity of NaOH used; NaOH; Volume of NaOH used; 0.09: Equivalent weight of lactic acid V: volume of juice.

2.6.4 Determination of Total Solids (TSS)

Total solids content was determined by weighing an empty filter paper and then passing a known weight of juice through a Whatman No. 1 filter paper that retained particle or solids. After drying in an oven (Fisher Isotherm 175) at 103°C for 2h. The solid left on the filter paper after evaporation was weighed and used to calculate the total solids. The total solids content is a measure of the amount of material remaining after all the water has been evaporated [15]. This is shown in the following equation.

$$\% \text{ Total solids} = (W_2 \times 100)/W_1 = (100 - \% \text{ moisture})$$

Where, W_1: Initial weight; W_2: Dried weight

2.6.5 Determination of Vitamin C

Vitamin C content was determined with the dichlorophenol-indophenol (DCP) method of Covenin and AOAC [16,20] with slight modifications.

2.6.5.1 Standardization of the DCP Titrant / mg Vitamin C Oxidized per 5 mL DCP Solution

9.7 mg of pure vitamin C ($C_6H_8O_6$) was accurately weighed out, dissolved with 50 mL distilled water and mixed enough to dissolve all of the ascorbic acid. 5 mL of the DCP was accurately pipetted into a 50 mL Erlenmeyer flask, 1drop of acetic acid (30%) was added to change the blue color of DCP to the pink color.

Using a burette, ascorbic acid solution was used to titrate the DCP till colorless endpoint (or equivalence point). The volume of ascorbic acid used was recorded and the titration repeated. The quantity of vitamin C that changed the colour of DCP was then calculated.

2.6.5.2 Titration of DCP with soursop or juice extracts

Standardization process was repeated by replacing ascorbic acid solution with 5mL of juice and made up to 10 mL with distilled water. After 2 times titration, the vitamin C content was calculated from volume of standard and expressed as mg ascorbic acid/100mL of juice.

2.6.6 Phytochemical analysis of formula

Both qualitative and quantitative analyses were carried out on soursop juice. The presence of major antioxidant secondary metabolite classes, namely; saponins, alkaloids, flavonoids, tannins, phenolics, and terpenoids were determined using standard phytochemical methods of Iqbal et al., *but* with some modifications [21].

2.6.6.1 Determination of Total Phenolic Content (TPC)

The total phenolic content of the juice was determined using the Folin-Ciocalteu colorimetric method as described by Prios et al. [22]. 0.2mL of each juice was added to test tubes containing 1.5mL of Folin-Ciocalteu reagent and incubated at room temperature for 5 minutes. Following this, 1.5mL of 6% sodium carbonate solution was then added to the mixture and re-incubated at room temperature for 90 minutes. The absorbance of the resulting blue colour was measured at 725nm using a quartz cuvette. Standard calibration curve for gallic acid in the range of 0–200µg/mL was prepared in the same manner and results were expressed as mg gallic acid equivalent (GAE) per mL of juice.

2.6.6.2 Test for saponins (Frothing test)

Saponins were tested by dissolving 0.5mL of the juice in a test tube containing 3mL of hot distilled water. The mixture was shaken vigorously for 1 minute to observe for persistent foaming [23].

2.6.6.3 Test for flavonoids (Cyanidine test)

0.5mL of the juice was dissolved in 2mL methanol and 1mL of concentrated sulphuric acid added. A spatula full of magnesium chloride ($MgCl_2$) powder was added and the mixture observed for effervescence. This was allowed for 1 minute to observe for a brick red colouration [24].

2.6.6.4 Test for steroids (Lieberman-Burchard test)

1mL of each juice was dissolved in 2mL of chloroform. Three drops of acetic anhydride were added to the test tube and boiled in a water bath for 10 minutes. It was rapidly cooled under running tap water. 2mL of Concentrated H_2SO_4 was then added along the side of the test tube. It was observed for the development of a greenish colouration [25].

2.6.6.5 Test for tannins (Ferric chloride test)

0.5mL of the juice sample was added to a test tube containing 20mL of boiling distilled water and then boiled for an hour. Five drops of ferric chloride were added and allowed to stand for proper colour development. A blue-black colouration indicated the presence of tannins.

2.6.6.6 Test for Alkaloids (Wagner's test)

0.5mL of each juice was separately stirred with 0.2mL of 1% HCl in a water bath for 5 minutes and filtered (Whatman™, 1002-147). 2g of Potassium iodide and 1.27g of iodine were dissolved in 5mL of distilled water and the solution was diluted to 100 mL with distilled water. Two drops of this solution were added to the filtrate; a brown coloured precipitate indicated the presence of alkaloids [25].

2.6.6.7 Test for cardiac glycosides (Keller-Killiani test)

0.5mL of each juice was added to 2 mL of glacial acetic acid containing one drop of 5% ferric chloride followed by 1 mL of concentrated sulphuric acid. It was observed for the appearance of violet and brownish rings below the interface, followed by the formation of a greenish ring in the acetic acid layer [26].

2.6.6.8 Test for phenolics

To 1 mL of each juice, one drop of 5% $FeCl_3$ was added. This was allowed to stand for 90 minutes and observed for the formation of a greenish precipitate [26].

2.6.6.9 Test for Terpenoids (Salkowski test)

0.5mL of each juice was mixed with 0.2mL of chloroform. 0.3mL of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inter face formed indicated the presence of Terpenoids [26].

2.7 Statistical Analysis

Values represented are the means and standard deviations for duplicate determinations. Statistical Analysis was carried out by Excel Version 10.0 software. Significance was defined at P =0.05 using the Bonferroni test in the Graphpad software version 6.

3. RESULTS AND DISCUSSIONS

3.1 Proximate Composition of Pulp and Pulp Extracts

Proximate composition of soursop pulp and concentrates (extract) are shown on Table 2. The pulps were significantly (P<0.05) higher than the concentrate (extract) in protein, lipid, fibre, ash and carbohydrate. The concentrates were higher in moisture than the pulp. Concentrate from day 5 ripening was significantly (P<0.05) higher than day 3 ripening in protein, and carbohydrate but significantly lower in moisture content. Lipid and ash were also lower but not significant.

3.2 Mineral Content of Pulp and Pulp Concentrates

The mineral content of soursop pulp and pulp concentrates are shown on Table 3. All samples were rich in the 7 minerals analysed with K (7.70 to 10.06 mg/g) being the highest concentration and Zn the least with a concentration range of 0.04 to 0.08mg/g. Comparatively, content, Ca and Na for pulp (P_5) and corresponding concentrate (EP5) were relatively constant while for pulp (P_3) and concentrate (EP3) the minerals whose concentration did not change are k, Fe, Zn and Na.

3.3 Sensory Evaluation of the Formulated Juices

The data for sensory analysis of formulated juice is shown on Table 4. The values for the overall acceptability of the juice ranged from 3.87to 6.38, with EP3F3 and EP5F3 having the highest overall acceptability; and EP3F2 and EP5F4 the least respectively for 3 and 5 days postharvest ripening. However, generally the differences in the sensory analysis of the formulated juices of day 5 ripening were not statistically significant (P>0.05). These two formulae (EP3F3 and EP5F3) with higher acceptability were therefore chosen for further work based on their sensory scores.

Organoleptic parameters like colour, flavour, and taste of the formulated soursop juices were acceptable throughout the period of storage except for juice stored at room temperature for more than 5 days which showed deterioration and fungi development. EP3F2 and EP5F2 exhibited the least acceptable scores in taste, flavour and overall acceptance after the 3rd and 5th day of storage. There were variations in colour and flavor in juice prepared with the three coloured water (additives).

3.4 Physicochemical Properties of the Formulated Juice

From the results of sensory evaluation, four juices were selescted for physicochemical analyses. pH, Total Solids (TSS) and Total Titratable Acidity (TTA) of the 4 selected juices are shown on Table 5. The results revealed that:pH value decreased in all four formulations and coincided with the increase in TTA. The initial pH of soursop pulp concentrate (EP3F1) was 5.04 and decreased to 3.42 after 5 days ripening (EP5F1). A similar trend was also seen in formulations EP3F3 and EP5F3.

Total Soluble Solids TSS increased during the ripening process of soursop from 3 to 5 days postharvest storage. The control formulation (EP3F1) reached a maximum of 11.08% after 3 days and 12.31% after 5days (EP5F1). The juice formulated with additives exhibited the same tendencies.

The Titratable Acidity of the formulated fruit juices at 25°C (Table 5.) increased significantly (at P< 0.05) during the storage period from 0.54 meq of Lactic acid/100mL juice EP3F1 to 0.72 meq lactic acid/100 mL of formulated juice EP5F1. This result is consistent with those observed` in the juice with additives (EP3F3 and EP5F3).

Table 5 shows that the vitamin C content of the formulated soursop juice increased significantly (P< 0.05) with increase in the postharvest ripening days. The content was high with fruits stored for 5 days compared to

those stored for 3 days. The vitamin C content was lower in juice formula EP3F3 and EP5F3 compared to EP3F1 and EP5F1 probably due to dilution effect of additives or the fact that low volumes of pulp concentrates were used.

3.5 Phytochemical Analysis

Qualitative phytochemical data of soursop juice is shown in Table 6. Qualitatively the juices presented with 6 phytochemicals. There were no steroids and glycosides recorded.

3.6 Quantitative Phenolics

Quantitative phenolic content is presented in Table 7. The table revealed that specifically, Juice EP3F1 had the highest while EP3F3 had the lowest total phenolic content because of low volume of pulp concentrate and dilution effect. TPC decreased significantly (at P> 0.05) with ripening duration for each formulation that is observed between day 3 (EP3F1) and day 5 (EP5F1). However, there was no significance difference between day 3 and 5 ripening.

Table 2. Proximate composition of soursop pulp and the pulp concentrate

Parameter (%)	Pulp (P3)	Pulp (P5)	EP3	EP5
Protein	5.35±1.40^{a}	7.45±0.53^{a}	0.58±0.01^{c}	3.23±0.02^{b}
Lipid	0.74±0.00^{b}	0.10±0.00^{a}	0.19±0.00^{a}	0.16±0.00^{a}
Fibre	6.26±2.00^{a}	24.23±4.00^{b}	1.26±0.09^{c}	2.56±0.06^{c}
Ash	1.83±0.01^{a}	2.22±0.06^{a}	1.62±0.01^{b}	1.29±0.00^{b}
Carbohydrate	20.62±3.00^{a}	21.00±1.00^{a}	8.63±1.40^{c}	13.89±0.40^{b}
Moisture	65.14±5.00^{a}	45.00±1.40^{b}	88.23±4.00^{c}	78.87±2.00^{c}

a, b, c: Values with same superscript on the same line are significantly different (P < 0.05). P_3 and P_5 = Pulps for day 3 and 5 ripeness respectively, EP3 and EP5 = Pulp concentrates for day 3 and 5 ripeness respectively

Table 3. Mineral content of soursop pulps and extracted juices

Parameter (mg/g)	Pulp (P3)	pulp (P5)	EP3	EP5
K	10.06	7.70	10.06	8.19
Fe	0.23	0.19	0.25	0.42
Ca	0.96	1.96	2.16	1.96
Mg	0.19	0.44	0.19	0.68
Zn	0.05	0.04	0.05	0.08
P	0.54	0.05	1.23	1.03
Na	0.73	0.73	0.81	0.73

P_3 and P_5 = Pulps for 3 and 5 days ripening respectively, EP3 and EP5 = Juice extracts from 3 and 5 days ripening respectively

Table 4. Mean Sensory Evaluation Score for formulated soursop juice

Day (Ripening)	Juice code	Colour	Taste	Flavour	Texture	Overall acceptability
3	EP3F1	5.36	2.64	3.27	4.91	3.95±1.30^{a}
	EP3F2	4.82	3.18	3.00	4.91	3.87±1.03^{a}
	EP3F3	7.09	4.82	4.82	6.27	5.62±1.13^{b}
	EP3F4	5.55	5.45	5.36	5.82	5.40±0.20^{b}
5	EP5F1	7.36	5.00	5.36	5.91	5.80±1.04^{b}
	EP5F2	5.82	4.55	5.27	5.18	5.11±0.52^{b}
	EP5F3	7.64	6.09	6.73	6.00	6.38±0.76^{b}
	EP5F4	6.00	5.64	6.00	5.18	5.62±0.39^{b}

Overall acceptability are Mean ± Standard Deviation of colour, taste, flavor and texture. Values with different superscript are significantly different (P < 0.05) for these acceptabilities

Table 5. Means physicochemical characteristics of the formulated juices

Parameter	EP3F1	EP5F1	EP3F3	EP5F3
pH	5.04±0.05[a]	3.42±0.03[a]	2.01±0.06[b]	1.39±0.09[b]
%TSS	11.08±2.00[a]	12.31±1.02[b]	4.44±0.00[c]	4.90±0.00[d]
TTA (lactic acid/100mL)	0.54±0.02[a]	0.72±0.05[a]	0.23±0.00[b]	0.28±0.01[b]
Vitamin C (mg/100mL)	27.50±5.05[a]	76.20±7.02[a]	11.01 ±0.71[b]	30.43±2.05[b]

On the same row means value between day 3 and 5 bearing different superscript letters were significantly different at P< 0.05

Table 6. Phytochemical Screening of formulated soursop juice

Parameter	EP3F1	EP5F1	EP3F3	EP5F3
Alkaloids	++	++	+	+
Saponins	++	++	+	+
Terpenoids	++	++	+	+
Steroids	-	-	-	-
Glycosides	-	-	-	-
Phenols	++	++	+	+
Tannins	++	++	+	+
Flavonoids	++	++	+	+

++ = Highly present ; + = Present, - = Absent; EP3F1 (Day 3, ripening with no additive); EP5F1 (Day 5, ripening with no additive); EP3F3 (Day 3, ripening with additive); EP5F3 (Day 5, ripening with additive)

Table 7. Total phenolic content of formulated soursop juice

Sample code	Total phenolic content (mg GAE/mL)
EP3F1 (Day 3, ripening with no additive)	0.89±0.01[a]
EP5F1 (Day 5, ripening with no additive)	0.53±0.23[b]
EP3F3 (Day 3, ripening with additive)	0.28±0.09[c]
EP5F3 (Day 5, ripening with additive)	0.26±0.00[c]

GAE = Gallic acid equivalent, M±SD = Mean ± Standard Deviation. Values with same superscript are not significantly different (P < 0.05) according to the Bonferoni test. EP3F1 (Day 3, ripening with no additive), EP5F1 (Day 5, ripening with no additive), EP3F3 (Day 3, ripening with additive), EP5F3 (Day 5, ripening with additive) (n=2).

4. DISCUSSION

Soursop pulp and juices formulations mde from them are rich in nutrients such as proteins, lipid, fibre, ash and carbohydrates. Protein content of soursop pulp is similar to those reported for important cereals which contain, in general, 7.8 to 22.8% [27,28] and higher than those from locust bean pulp (4.29%) [29]. Although the protein content of the formulated juices were lower than reported values, the pulp can be a potentially good source of proteins which should be exploited commercially.

Crude fat represents the true fat and other materials such as phospholipids, sterols, essential oils and fat soluble pigments in the fruit. Generally fruits have low levels of fat. This study recorded low fat content in soursop fruits comparable to the value of 0.31% for soursop fruits reported by Moos, [30]. The low level of fat in the fruits could make valuable means for loss or maintenance of weight, and lowering of blood pressure [31-32].

This study recorded a fibre value higher than the values of 1.6% for soursop fruit (pulp) reported by Moos [30] and equally higher than 11.5% reported for soursop from Ghana [33]. Fiber is essential to the human body as it helps to maintain the health of the gastrointestinal tract and in weight regulation [34], but if consumed in excess it may bind trace elements, leading to deficiency of iron and zinc in the body [34]. The observed level of crude fibre in Cameroon soursops is moderate and thus good for body maintenance.

Ash is the inorganic residue remaining after heating to remove all the water and organic matter and it provides a measure of the total amount of minerals in a food. The main purpose of ash determination is to assess the quality of the food minerals. The ash content in this study

is higher than that reported by Moos [30] which was 0.73, but compares well with 2.44% reported by Boakye [33] for soursop from Ghana. The results in this study thus suggest that the fruits have high deposits of mineral elements. Soursop fruit and consequently juice analyzed were rich in potassium, iron, calcium, magnesium, zinc, phosphorus and sodium, with potassium having the highest concentration (7.70 to 10.06mg/g) and zinc the least (0.04 to 0.08mg/g). Minerals are important in human nutrition. It is well known that enzymatic activities as well as electrolyte balance of the blood fluids are related to adequacy of Na, K, and Mg. Potassium is very important in maintaining the body's fluid volume and osmotic equilibrium. The high values for calcium, phosphorus, and magnesium observed in the fruits indicate that they can play a vital role in the development of bones, teeth, co-factors in enzymatic reactions, nerve impulse transmission and also blood clotting [35]. The consumption of these fruits will therefore help to improve wellbeing [36].

Carbohydrate is the highest macronutrient present in soursop fruit. Carbohydrate content is higher than those from locust bean pulp (6.28%) [29]. The most abundant component of soursop is water; but as the day of storage increases the moisture content decreases. The high moisture content is beneficial as it makes the fresh fruit juicier and more palatable to the consumer. Thus for preservation and diversification purposes, it would be necessary to process the fruit pulp into juice.

In this study the sensory analysis of the formulated juices showed that EP3F3 (juice with additives of day 3 soursop ripening) was the most acceptable. Its preference may be due to the fact that it had additives which masked the natural flavour of soursop as compared to EP3F1 and EP5F1 which are formulated without additives. Some people may not quite appreciate or like the natural soursop flavour. However, generally the differences in the sensory analysis of the formulated juices of day 5 ripening were not statistically significant ($P>0.05$). The vanilla sugar, aroma essence and soursop pulp concentrate had significant effects ($P<0.05$) on the taste, color acceptability, and the intention to accept the soursop formulations. A higher percentage of sugar led to higher acceptability of taste, color, and intention of purchase. Whereas the amount of pulp concentrate had an opposite effect, a higher pulp concentrate, resulted in lower acceptability of taste, color, and intention of purchase. Similar results were presented by Utomo et al. [37], who prepared a fruit leather to evaluate the acceptability of different soursop pulp. This rejection is due to a bitter taste in the soursop, caused by organic acids (malic acid, citric acid, and isocitric acid) present in the fruit [37,38].

The decrease in pH observed with ripening was also reported by Paull [39] for soursop. This decrease is attributed to an accumulation of H^+ ions due to the larger quantity of supplemental organic acids which can be found in non-ionized form, and when released from the vacuole, remain in the cytoplasm and are measured as free H^+-ions in the fruit pulp [40].

The increase in TSS in this study may have been caused by starch hydrolysis, sucrose, pectins and other soluble compounds such as organic acids or amino acids. Lima et al. [41] found an increase in the hydrolysis of starch and pectins of soursop after 3 days of storage at 26°C. A value of 17.65 °Brix is reported for soursop when fully ripe [42]. Paull [39] also noted that TSS of soursop increased to about 16 °Brix when fully ripe (day 3).

The increase in titratable acidity with ripening duration can be explained by the observation of Paull et al. [43] who reported that in ripe soursop, malic acid, citric acid or lactic acid increased seven fold and citric acid increased threefold between days 3 and 4 postharvest, and then decreased. Another acid that can intervene in soursop acidity is ascorbic acid, as it has been reported to increase 11-fold during the ripening process [39]. According to the FAO [42] the acidity of soursop when ripe is 0.85 meq malic acid/100 g Fresh weight and coincides with our results.

The increase of vitamin C with ripening day is of great health significance and implies that the juice can take care of vitamin C deficiency related ailment like scurvy [44]. The results obtained here are consistent with the observation of Cluter and miller [45] who reported that faster ripening fruits contained significantly more ascorbic acid when ripe than did slower ripening ones. In addition, their results indicate that ascorbic acid increases in the ripening of detached fruits.

Sensory analysis shows EP3F3 to be the most acceptable, this could probably be due to the fact that most of the tasters were youths who

appreciate sweet things to non-sweet; EP5F1 was formulated from just the pure pulp concentrate with no additives whereas EP3F3 had additives.. This could equally be due to the fact that some people do not appreciate the flavour of natural soursop fruit. However, there was no significant difference in the sensory analysis amongst the formulated juice at day 5 ripening ($P>0.05$).

Phytochemical screening of the formulated juice of soursop revealed the global richness of the juice in secondary metabolites. The phytochemical compounds detected are known to have medicinal importance. For example, many alkaloids from plants have been reported to show biological activities such as, anti-inflammatory, antimalarial, antimicrobial, cytotoxicity and pharmacological effects [46-49]. Tannins, are known to have antibacterial [50], antitumor and antiviral activities [51]. These phytochemical compounds identified in the juice could be important for their use in traditional medicine.

It is proposed that pulp and skin darkening is a symptom of chilling injury, which is due to increased activity of polyphenoloxidases on phenolic compounds that exist in skin and pulp [13]. Lower accumulation of total phenolics with postharverst storage, can be explained by reduced activity of polyphenoloxidase and peroxidase, which are involved in the oxidation of phenolic compounds [13]. It is reported that Annona fruits are a source of phenolic compounds. For example, *Annona muricata L*. has 624.2-941.4 mgGAE mg-1 pulp [52] and *Annona squamosa* 583.45 µg catequina mL^{-1} [53].

Natural polyphenols are secondary metabolites produced by plants for their defense against different types of stress, e.g. ultraviolet radiation, aggression of pathogens, low soil fertility, changes of environmental temperature, severe drought, and grazing pressure [54]. The interest on plant phenols is increasing in the recent decade because of their health promoting potentials. They constitute a class of antioxidants which have protective effects against a variety of diseases, particularly cardiovascular disease and cancer. The lowest concentration of EP5F3 ripening indicates that as the day of ripening increases the total phenolic content decreases due possibly to chloroplast breakdown releasing polyphenol oxidases causing oxidation and polymerization of the phenols. The decline in phenols may probably lead to a loss of astringency during ripening leading to a bland flavour of the slightly overripe fruit [55]. This may also explain why very ripe fruit have off-flavour which may be due to very low phenols [39], lower organic acids [43] and some fermentation [47]. It can be suggested that the synergistic effects of phytochemicals present in this plant juice may be the underlying principle behind the chemotherapeutic potentials reported for soursop.

5. CONCLUSION

The results of the analyses on the pulp and juice of *Annona muricata* shows that it is rich in nutrients such as protein, lipid, fibre, ash, carbohydrate and minerals K had the highest mineral concentration (7.70-10.06mg/g) and Zn the least (0.04-0.08mg/g). Juice EP3F3 was preferred followed by EP5F3 as compared to EP3F1 and EP5F1 probably because EP3F3 and EP5F3 had additives. Six phytochemicals (Alkaloids, Saponins, Terpenoids, Phenols, Tannins and Flavonoids) were detected in the fruit pulp and juice which may be used for traditional medicine. Both ascorbic acid and total polyphenol content make the soursop a product with potential health characteristics with agro-industrial applications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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