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Investigation on nutritional potential of soursop (*Annona muricata* L.) from Benin for its use as food supplement against protein-energy deficiency

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Abstract

The microbiological and nutritional characterization of Soursop pulp (*Annona muricata L.*) was investigated. The moisture content was ranged from 18.33 to 24.53%. The pH was between 4.1 and 4.8 with a mean acidity of 1.75%. The soursop pulps are rich in nutrients such as carbohydrates (23.05%), proteins (7.41%), ash (2.22%) and fiber (24.73%). All samples analyzed were rich in minerals such as phosphorus, calcium, magnesium, potassium and sodium, with a higher content of potassium (1.29 to 1.35%). Anti-nutritional factors such as oxalate and phytate were detected in samples, and values were lower than established toxic level. The total flora count of samples from markets ranged from 2×10^1 to 7×10^1. The enumeration of total coliforms and fecal coliforms was less than 10 cfu/g with an absence of pathogens. The results of physicochemical parameters of soursop puree during storage shown that moisture content, pH and acidity were 21.53±0.14%, 4.1±0.03 and 0.22±0.01%. These physicochemical parameters were significantly ($p < 0.05$) influenced by the storage time in unpasteurized puree. However, in pasteurized puree, the stability of physicochemical parameters is observed for 15 days of storage at 25°C. However, due to the fact that the soursop fruit is rich in nutrient and have high moisture content, which would encourage microbial growth and so deterioration, more attention should be paid to its microbial quality.

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Introduction

Edible wild indigenous plants become an alternative source of food with high potential of vitamins, minerals and others interesting elements particularly during seasonal food shortage (Umaru *et al.,* 2007). Wild fruits are also known to have nutritional and medicinal properties that can be attributed to their antioxidant effects and they can be used to fortify staple foods particularly for malnourished children (Barminas, 1998). There is much evidence that the quality and composition of food may contribute to present and future health benefits of young children. According to the World Health Organization, malnutrition is the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions (Tierney et *al*., 2010). The term protein-energy malnutrition (PEM) applied to a group of related disorders that include marasmus, kwashiorkor, and intermediate states of marasmus-kwashiorkor (Tierney et *al*., 2010). The most common form of malnutrition in Africa is protein energy deficiency affecting over 100 million people, especially 30 to 50 million children under 5 years of age (Jildeh et *al*., 2010). Some legumes such as soybean, bean, and peanut, are important sources of protein and can therefore help to increase the protein intake of the diet of population. However, the low-income group, especially in rural areas, sometimes cannot afford these protein foods. Soursop (*Annona muricata*) belongs to the family Annonaceae, and it is wide spread in the tropics and frost–free subtropics of the world (Samson, 1980). The soursop plant is cultivated mainly in home gardens. The tree yields up to 10 tons/ha and each fruit weighs 0.5 to 2 kg (National Academy of Science, 1978). The fruit is compound in and covered with reticulated, leathery-appearing but tender, inedible bitter skin from which protrude 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, subacid to acid flavor is unique (Schultes and Raffauf, 1990). It is indigenous to most of the warmest tropical areas in 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. The 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’ use (Ayensu, 1981). All parts of the *A. muricata* tree are used in natural medicine in the tropics including the bark, leaves, root and fruit-seeds. The crushed seeds are used as a vermifuge and anthelmintic against internal and external parasites and worms. The bark leaves and roots are considered sedative, antispasmodic, hypoglycemic, hypotensive, smooth muscle relaxant and nervine and a tea is made for various disorders for those purposes (Holdsworth, 1990). Many bioactive compounds and phytochemicals have been found in *A. muricata* and its many uses in natural medicine have been validated by many scientific researches (Heinrich, 1992; Sundarrao, 1993). Generally the fruit and fruit juice is taken for worms and parasites, to cool fevers, to increase mother’s milk after childbirth (lactagogue), and as an astringent for diarrhea and dysentery. Due to the fact that the fruit has a wide distribution throughout tropical and subtropical parts of the world and mainly in developing country, the aim of this study was to investigate the physicochemical, the nutritional potential and mycoflore associated with Soursop (*Annona muricata L.*) from Benin. An emphasis has been placed on its use as food supplement against Protein-Energy Deficiency. For its valorization, the physicochemical and microbial changes in soursop puree which has been pasteurized at optimum thermal condition (Umme et *al*., 1997) were also investigated. This study also aims to make available an improved industrial technology for the production of soursop puree, which retains all the nutritional potential of the fruit during storage.

Materials and methods

Collection of samples

Samples (fruit of *Annona muricata*) were purchased from local markets (the major sales depot of *A.*

muricata) in Sekou, Akassato, and Abomey-calavi, all in South of Benin and labeled A, B, and C respectively. The samples were purchased from four different points in each market and were mixed together to give each composite sample which was used for the analysis. Fresh fruits were also harvested after maturation and taken to the laboratory where they were dried at laboratory temperature (25°C). Husks were manually removed. The pulp were collected and kept in airtight container for laboratory analysis.

Préparation of puree

Fully ripe soursop (*A.muricata* L.) fruits were washed under running tap water, hand-peeled, cored, deseeded and the pulp macerated. Water was added in the ratio of 1:2 (w/v, pulp:water) to facilitate the maceration process and it was repeated twice to achieve a smooth-texture puree. The soursop puree was pasteurised at optimum conditions of 79°C for 69 s and rapidilly cooled to 9°C. The cooled puree was packed into sterile container. Unpasteurised soursop puree were also made. All samples were stored at 25°C for periodical analyses.

Determination of physicochemical parameters

Moisture content of samples was determined by desiccation using the method of De Knegt and Brink (1998). A clean platinum dish was dried in an oven and cooled in a desiccator and weighed. From each sample, 5 g was weighed and spread on the dish, the dish containing the sample was weighed. It was then transferred into the air oven at 105°C to dry until a constant weight was obtained and the loss in mass was determined. In order to obtain the pH of the samples, 5 g of each sample was weighed, grinded and suspended in10 ml of distilled water. The pH was determined with a digital pH-meter (HANNA HI 98129). Acidity of samples, expressed as citric acid content per unit of volume, was determined by titration with 0.01 mol/L of sodium hydroxide solution, using phenolphthalein as indicator (AOAC, 1995).

Nutritional analysis

The carbohydrate was determined according to phenol sulfuric acid method (Agbo and Ronald, 1996; Ezoua et *al*., 1999). A standard curve was obtained using the following concentration of sucrose in (mg/ml) 2.5 2.0, 1.25, 1.0, 0.5 g of each sample with 9 ml of distillated water was measured into test-tube. 2 ml of phenol solution (1%) and 1 ml of concentrated H_2SO_4 solution were added. This was shaken for 15 min and boiled for 30 min. It was then allowed to cool. The absorbance was then read off a spectrophotometer (Spectrum lab 22) at 700 nm. The sugar concentration was then obtained by extrapolation from the standard curve. Protein was analyzed by the Microkjedhal nitrogen method, using a conversion factor of 6.25 and fat content was obtained by Soxhlet extraction as described by Pearson (1976). Ash was determined according to the standard methods described by the Association of Official Analytical Chemists (AOAC, 1990). Minerals were analyzed by the method reported by Oshodi (1992). Minerals were analyzed by dry-ashing 1 g of the sample at 550°C in a furnace. The ash obtained was dissolved in 10% HCl, filtered with filter paper and made up to standard volume with deionised water. Flame photometer was used to determine potassium content of the samples, while calcium and magnesium were determined using atomic absorption spectrophotometer (Perkin Elmer, Model 403).

Anti-nutritional factors analysis

Total oxalate was determined as described by Day and Underwood (1986). 1 g of sample was weighed into 100 ml conical flask. 75 ml H_2SO_4 (3 mol/L) was added and stirred for 1 h with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. 25 ml of the filtrate was then taken and titrated while hot against 0.05 mol/L of $KMnO_4$ solution until a faint pink colour persisted for at least 30 s. The oxalate content was then calculated by taking 1 ml of 0.05 mol/L of $KMnO_4$ as equivalent to 2.2 mg oxalate (Ihekoronye and Ngoddy, 1985; Chinma and Igyor, 2007). Phytate was determined using the method of Reddy and Love (1999). 4 g of each sample was soaked in 100 ml of 2% HCl for 5 h and filtered. To 25

ml of the filtrate, 5 ml of 0.3% ammonium thiocyanate solution was added. The mixture was then titrated with Iron (III) chloride solution until a brownish-yellow color that persisted for 5 min was obtained. A 4:6 Fe/P atomic ratio was used to calculate the phytic acid content (Okon and Akpanyung, 2005).

Microbiological analysis

To 25 g of each sample, 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated at 30°C for 72 h. Desoxycholate was used for the total Coliforms count and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the Faecal coliforms count. In this case, plates were incubated at 44°C and the identification was made using EMB (Eosine Methylene blue). Tryptone Sulfite Neomicin Agar was used for Anaerobic Sulfito-Reducer (ASR) count and tubes were incubated at 37°C for 24 h. After incubation, the number of colonies was tracked using a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, bearing in mind the factors of dilution (Singh et *al.*, 1991). The isolation of fungi from samples was performed using dilution plating method. 10 g of each sample were separately added to 90 ml of sterile water containing 0.1% peptone water. This was thoroughly mixed to obtain the 10–1 dilution. Further 10-fold serial dilutions up to 10–4 were made. One mililitre of each dilution was separately placed in Petri dishes, over which 10 to 15 ml of Potato Dextrose Agar with 60 µg/ml of chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2°C for 7 days (Rampersad et *al.*, 1999).

Table 1. Physicochemical parameters of soursop pulp.

Samples	Moisture (%)	pH	Acidity (%)
A	22.53±0.14[a]	4.1±0.03[a]	2.1±0.01[a]
B	18.33±0.12[a]	4.6±0.02[a]	1.7±0.01[a]
C	24.53±0.01[a]	4.8±0.02[a]	1.3±0.01[a]
D	21.03±0.26[a]	4.4±0.02[a]	2.2±0.01[a]
Fresh pulp	19.53±0.17[a]	5.2±0.02[a]	1.2±0.01[a]

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 2. Nutritional content of soursop pulp.

Samples	Carbohydrate (%)	Protein (%)	Ash (%)	Fiber (%)
A	21.0±0.03[a]	7.45±0.05[a]	2.21±0.67[a]	24.23±0.14[a]
B	23.2 ± 0.85[a]	7.41± 0.05[a]	2.25±0.73[a]	27.28±0.11[a]
C	25.0 ± 0.62[a]	7.87± 0.32[a]	2.19±0.27[a]	22.17±0.18[a]
D	25.1 ± 0.53[a]	7.37± 0.51[a]	2.24±0.61[a]	24.21±0.16[a]
Fresh pulp	26.0 ± 0.37[a]	7.91± 0.12[a]	2.29±0.53[a]	24.32±0.10[a]

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Statistical analyses

The data generated from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA) (Alder and Roessler, 1977; Ogbeibu, 2005).

Results

The results of physicochemical characterization of different samples of soursop pulp from markets (Table 1) showed that the moisture content of different samples ranged from 18.33 to 24.53%, with an average of 21.43%. The pH was between 4.1 and

4.8 with a mean acidity of 1.75%. The soursop pulps are rich in nutrients (Table 2) such as carbohydrates (23.05%), proteins (7.41%), ash (2.22%) and fiber (24.73%). The analysis of anti-nutritional factors revealed the presence of oxalate (6.45 to 6.53%) and phytate (2.72 to 2.79%) (Table 3). All samples analyzed were rich in minerals such as calcium, magnesium, potassium and sodium, with a higher content of potassium (1.29 to 1.35%) (Figure1). The result of proximate composition of fresh pulp of soursop is as shown in Table 4. The moisture content and acidity were respectively 19.53 ± 0.17 and 1.02 ± 0.01%. Ash, protein and carbohydrate content were 2.19 ± 0.27, 7.91 ± 0.12 and 26.0 ± 0.37%, respectively. Fiber content was 24.32 ± 0.10%. All samples analyzed were also rich in minerals such as phosphorus, calcium, magnesium, potassium and sodium, with a higher content of potassium (1.3 ± 0.22%) (Fig. 1).

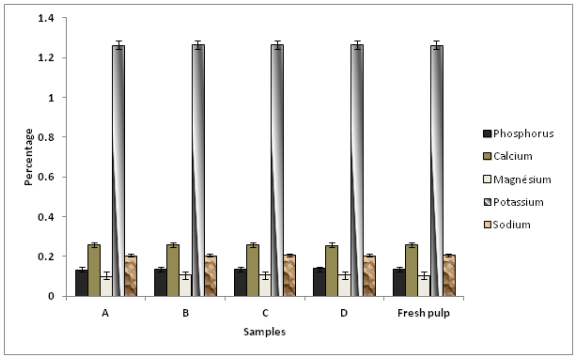

Fig. 1. Mineral content of soursop pulp.

Table 3. Antinutritional factors content of soursop pulp.

Samples	Oxalate (%)	Phytate (%)
A	6.45 ± 0.43[a]	2.79 ± 0.48[a]
B	6.53 ± 0.49[a]	2.72 ± 0.76[a]
C	6.48 ± 0.41[a]	2.76 ± 0.37[a]
D	6.51 ± 0.46[a]	2.73 ± 0.65[a]
Fresh pulp	6.57 ± 0.42[a]	2.78 ± 0.37[a]

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

The analysis of anti-nutritional factors also revealed the presence of oxalate (6.57 ± 0.42%) and phytate (2.78 ± 0.37%) (Table3). Physicochemical analysis of pulps coming from different regions did not shown significant statistical difference ($p < 0.05$) between samples. The total flora count of samples from markets ranged from 2×10^1 to 7×10^1. The enumeration of total coliforms and fecal coliforms was less than 10 cfu/g with an absence of spores of anaerobic sulfite reducers (ASR). The microbial contamination of fresh pulp was very low with the absence of pathogens (Table 4). The results of physicochemical parameters of soursop puree during storage are presented in Table 5. The moisture content, pH and acidity of the soursop puree were 21.53± 0.14%, 4.1± 0.03 and 0.22±0.01%.These physicochemical parameters were significantly ($p < 0.05$) influenced by the storage time in unpasteurized puree. However, in pasteurized puree, the stabilization of physicochemical parameters is observed for 15 days of storage at 25°C. The results of microbial analyses of soursop puree also indicated the growth of bacteria and fungi in unpasteurized puree unlike to pasteurized puree. However, the growth of bacteria and fungi were also observed in pasteurized puree after 15 days of incubation at 25°C.

Discussion

The results of the proximate composition revealed that soursop fruit are good source carbohydrates, proteins, and crude fibers. Protein content of soursop pulp is similar to those reported for important cereals which contain, in general, 7.8 to 22.8 g / 100 g (Bullock *et al.*, 1989; Ranhotra *et al.*, 1996) and higher than those from locust bean pulp (4.29%) (Dahouenon-Ahoussi et *al.*, 2012). Thus, soursop fruits are a potentially good source of proteins which should be exploited to determine if they are commercially viable. These fruits can also play vital role as supplementary nutrient source to cereal and other low nitrogen farm products currently used in feed formulation. Carbohydrate is the highest macronutrient present into soursop fruit. Its value is higher than those from locust bean pulp (6.28%) (Dahouenon-Ahoussi et *al.*, 2012). Crude fibers are also present in the soursop fruit. Although crude fiber does not contribute to nutrients or energy, it is a

source of dietary fiber. This value of fiber might be helpful in terms of maintaining positive effects on intestine and colon physiology, besides other homeostatic and therapeutic functions in human nutrition (McPherson, 1982). Minerals are important in human nutrition. It is well known that enzymatic activities as well as electrolyte balance of the blood fluid are related to adequacy of Na, K, and Mg. Potassium is very important in maintaining the body fluid volume and osmotic equilibrium. Metal deficiency syndrome like rickets and calcification of bones is caused by calcium deficiency. The high values of calcium, phosphorus, and magnesium observed in the fruits indicate that these fruits can play a vital role in the development of bones, teeth, co-factor in enzymatic reaction, nerve impulse transmission and as a clotting factor (Hatton and Mc carron, 1994). The consumption of these fruits will also help to alleviate symptoms of magnesium and zinc deficiency such as weakness, cardial arrhythmia, poor growth, impairment of sexual development. Several studies on nutrition in developing countries have shown that adequate nutrient intake (daily calories, daily protein, daily fat, minerals and vitamins) is an essential ingredient for improved well-being, economic growth and development, since a healthy body enhances the capacity to learn which in turn determines productivity and economic growth (Flores, 2001; Diao et *al*., 2007). Anti-nutritional factor such as oxalate and phytate are also present in the fruit. Its content is lower than those from locust bean pulp (Dahouenon-Ahoussi et *al*., 2012).

Table 4. Microbial count of soursop pulp (cfu/g).

Samples	Total Bacterial Count	Total Coliforms count	Faecal Coliforms count	A.S.R spores count	Mould and Yeast count
Fresh pulp	07	00	00	00	01
A	2 x 10^1	01	00	00	01
B	6 x 10^1	08	00	00	05
C	4 x 10^1	05	03	00	02
D	7 x 10^1	07	02	00	02
European Union criteria (2005)	-	10	10	Absence/10g	Absence/10g
Conformity (%)	-	100	100	100	00

A.S.R: Anaerobic Sulfito-Reducer

Table 5. Evolution of physicochemical parameters of soursop puree during storage.

Storage days	Moisture		pH		Acidity (%)	
	Pasteurised	Unpasteurised	Pasteurised	Unpasteurised	Pasteurised	Unpasteurised
0	21.53±0.14^a	22.53±0.14^a	4.1±0.03^a	4.0±0.06^a	0.22±0.01^a	0,21±0.01^a
5	21.32±0.14^a	20.76±0.18^b	4.2±0.07^a	2.4±0.01^b	0.22±0.02^a	2.6±0.04^b
10	21.43±0.14^a	16.01±0.13^c	4.1±0.01^a	1.7±0.04^c	0.20±0.04^a	2.9±0.07^b
15	21.41±0.14^a	14.01±0.17^d	4.2±0.03^a	1.4±0.02^d	0.21±0.07^a	3.5±0.02^c
20	20.01±0.14^b	13.47±0.19^e	3.7±0.08^b	1.2±0.06^d	1.9±0.03^b	3.9±0.01^c
25	18.41±0.14^c	12.81±0.14^f	2.9±0.02^c	1.2±0.08^d	2.4±0.01^c	4.1±0.07^c
30	14.89±0.14^d	12.01±0.14^f	2.4±0.01^c	1.1±0.04^d	2.9±0.04^d	4.9±0.02^d

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 6. Evolution of microbial quality of pasteurized soursop puree during storage.

Storage days	Total Bacterial Count	Total Coliforms count	Faecal Coliforms count	A.S.R spores count	Mould and Yeast count
0	2×10^1	00	00	00	01
5	2×10^1	00	00	00	01
10	1×10^2	00	00	00	03
15	1.3×10^2	00	03	00	09
25	2.7×10^2	00	00	00	2.7×10^1
30	3.4×10^2	00	00	00	3.0×10^2
European Union criteria (2005)	-	10	10	Absence/10g	Absence/10g

A.S.R: Anaerobic Sulfito-Reducer

Table 7. Evolution of microbial quality of unpasteurized soursop puree during storage.

Storage days	Total Bacterial Count	Total Coliforms count	Faecal Coliforms count	A.S.R spores count	Mould and Yeast count
0	07	00	00	00	03
5	2×10^2	01	00	00	2.0×10^1
10	6×10^2	08	00	00	3.0×10^2
15	4×10^3	05	03	00	7.0×10^2
25	7×10^3	07	08	00	3.0×10^3
30	1×10^4	09	14	00	3.0×10^3
European Union criteria (2005)	-	10	10	Absence/10g	Absence/10g

A.S.R: Anaerobic Sulfito-Reducer

According to Oke (1969), oxalate can bind to calcium present in food thereby rendering calcium unavailable for normal physiological and biochemical role such as the maintenance of strong bone, teeth, cofactor in enzymatic reaction, nerve impulse transmission and as clotting factor in the blood. The calcium oxalate, which is insoluble, may also precipitate around soft tissues such as kidney, causing kidney stones. However, the values obtained for soursop pulp were below the established toxic level. Phytate diet of 1 to 6% over a long period decreases the bioavailability of mineral elements in monogastric animals. Phytic acid can bind to mineral elements such as calcium, zinc, manganese, iron and magnesium to form complexes that are indigestible, thereby decreasing the bioavailability of the element for absorption (Erdman, 1979). Phytic acid also has a negative effect on amino acid digestibility (Makkar and Becker, 1998). However, values obtained from these locust bean pulp powder samples are lower than established toxic level. The moisture content of the soursop pulp is quite high. This high moisture content would encourage microbial growth and so deterioration. These results are in agreement with those obtained in the determination of the microflora associated with soursop fruit. This microbial contamination was due to the high nutritional value of soursop fruit and is in relation with its rapid deterioration. The production of soursop puree in order to the valorization of the soursop fruit is then justified. However, the assessment of physicochemical parameters of purees during storage shown that the pasteurized puree is storable for 15 days in opposite to the unpasteurized puree. This stabilization of the pasteurized puree is due to the action of heat on the fermentative microorganisms. Unfortunately this inactivation is only effective for 15 days because there are later proliferations of microorganisms including fungi which are spore former and able to resist at relatively high temperature. These results are also confirmed by

the evaluation of microbiological parameters of purees during storage.

Conclusion

This survey underlined the nutritional potentiality of soursop pulp (*A. muricata*) for its use as food supplement against protein-energy deficiency. However, due to the fact that the soursop fruit is rich in nutrient, it high moisture content would encourage microbial growth and so deterioration. More attention (in the process and storage methods) should be paid to its microbial quality in order to preserve children health.

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