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Microbial Quality Analysis and Nutritional Composition of Soursop Juice Under Different Storage Conditions

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ABSTRACT

Soursop is a tropical fruit which shows a good potential for use in the formulation of a functional drink. It is highly susceptible to contamination, which can affect it shelf life and quality. The purpose of this research is to determine the microbial quality analysis and nutritional composition of soursop juice under different storage condition. Soursop juice sample was extracted from Soursop fruit for analysis. All samples were analysed for the density of microorganism present using standard plate count method. The dilution factor of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} was used for inoculation by taking 1000 ml of the soursop mixture (that is 1 gm of soursop in 9 ml distilled water) into each tubes of different dilution containing 10 ml of water. Total viable plate count of microorganisms, nutritional composition and vitamin composition of the soursop juice sample was carried out using a standard method. The microbial count of the soursop juice sample showed a significant difference ($p<0.05$) for bacteria count, coliform count, mold/yeast count. The microbial count of soursop juice for all samples revealed the total viable bacteria count of soursop juice before storage and after seven days storage ranged from 1.20×10^2 – TNTC cfu/ml, total coliform ($2.17 \times 10^2 – 26.13 \times 10^2$ cfu/ml) and total yeast/mold count (4.10×10^2 sfu/ml – TNTC). The species of bacteria isolated from the Soursop juice before storage were *Bacillus pumilus, Paenibacillus thailander*, and after storage in room and refrigerated temperature were *Staphylococcus arlettae, Staphylococcus tequilensis, Staphylococcus xylosus.*

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The results for proximate analysis (%) ranges were, moisture (63.10±0.03 -74.45±0.02c), ash (5.54±0.04 – 8.27±0.04), fat (0.63±0.01 – 1.07±0.04), fibre (0.12±0.00 – 0.17±0.00), protein (2.53±0.02 – 3.37±0.01) and carbohydrate (15.14±0.05 – 24.08±0.10). Also, the result of vitamin composition shows that Vitamin A content ranges from 315.16±48.11– 674.26±1.89, vitamin B1(1.22±0.00a – 2.68±0.01c), vitamin B2 (0.69±0.00a – 2.75±0.00d) and Vitamin C (36.98±0.06a – 71.01±1.64c). Storage temperature greatly affects the microbiological stability and hence the quality of the soursop juice. It is recommended that studies should be conducted on the effect of packaging and use of chemical preservatives on the storage stability of soursop juice. However, as part of recommendation studies should be conducted to reduce the susceptibility of the fruit to microbial spoilage and to ensure its effectiveness in different applications to preserve the shelf life.

Keywords: Soursop, microorganisms, microbial count, room temperature, refrigerated temperature, nutritional composition, vitamin composition.

1. INTRODUCTION

Annona muricata L. (*Soursop*) is a lowland tropical fruit-bearing tree in the Annonaceae family. *Annona muricata* is also commonly known as Graviola, Soursop, or Gunabana. The name soursop is due to sour and sweet flavour of its large fruit. According to Coria-Téllez *et al*. (2018), the main active components of *Annona muricata* are acetogenin, alkaloids, and flavonoids.

Barbalho *et al*. (2012) reported that Acetogenins (ACGs) are the major constituents of the Annona genera and examples were found to possess a variety of pharmacological properties including antitumor, immunosuppressive, pesticidal, antiprotozoal, antimicrobial, antimalarial, anthelmintic, and antiviral agents, with some being commercially developed treating infestations of head lice, fleas, and ticks. Analysis of the compounds in *Annona muricata* leaf extract revealed secondary metabolites such as flavonoids, terpenoids, saponins, coumarins, lactones, anthraquinones, glycosides, Tannins, and phytosterols (Gavamukulya *et al.,* 2014). Soursop phytochemicals have been used in herbal therapy for a long time. They can be used to treat medical conditions, such as bacterial or parasite infections, fever, hyperglycemia, hypertension, inflammation, anxiety, and cancer. According to Gyesi *et al.* (2019), the soursop extract has demonstrated antibacterial, antiprotozoan, anti-inflammatory, antioxidant, and antitumor properties in different scientific interventions.

The soursop fruit has a distinct aroma and flavour (Neta *et al.,* 2019). It Is a unique fruit with delightful fragrant and creamy pulp. The fruit is rich in vitamins, minerals, and dietary fiber (Olagunju *et al.,* 2018). The soursop Leaf was identified to be useful in inhibiting the cancer cells by inducing apoptosis, improving immune response, decreasing glucose concentration in blood, reducing depression, stimulating digestion, and dilating blood (Gajalakshmi *et al*., 2012). The soursop fruit carries the phenolic compounds and alkaloids which can lower BP and control the uric acid production in hyperuricemia patients (Nayak and Hedge, 2021). Ethanol extract of 96% of soursop leaves at concentrations can reduce total plasma cholesterol (Ramatillah *et al*., 2017). To decrease cholesterol levels, people take three to five doses of soursop leaf extracts daily, which are boiled in two to three glasses of water until just a half to one glass of water remains. In the Guianas, a tea is prepared from either the leaf or bark as a cardiotonic or sedative (Taylor, 2002). In the world today, many techniques and technologies have been developed to help increase the preservation time as well as the sensory quality of products in general and of fruits in particular (Ceballos *et al.,* 2012). Creating dried products from soursop will help preserve and increase the value of this fruit (Chang *et al.,* 2019; Chang *et al.,* 2018).

2. MATERIALS AND METHODS

2.1. Sample Materials

Fresh soursop fruits were purchased from Shasha Market and was transported to the laboratory. The research analyses were performed at Microbiology Department,Olusegun Agagu University of Technology Okitikputa, Ondo state.

2.2. Sample Preparation

The Soursop juice samples were collected into sterile polythene nylon and brought immediately to the laboratory under a sterile condition. Due aseptic care was taken during transportation, and the samples were kept from contamination until they were subjected to bacteriological analysis. The soursop was peeled, seed were removed, and pulp was removed thereafter for proper extraction of the juice. The seeded soursop was blended and sieved in cheesecloth to extract the rich, creamy juice.

2.3. Microbiological Analyses

2.3.1. Preparation and Inoculation of Samples

The pour plate method was used for culture. About 1.0 grams of the samples was weighed by taken aseptically with a sterile spatula and transferred carefully into each of the test tubes containing 9.0 ml of cooled sterilized water, each soil samples in different test tubes were mixed thoroughly to ensure dislodgement and even distribution of microorganisms into the suspended sterile water. A two-fold serial dilution of each 1ml homogenate was prepared. Exactly 1.0 ml of dilution factor 10^{-1} and 10^{-2} were inoculated into the sterile petri dishes for culturing. Incubation was carried out at 37°C for 24 hours for bacteria growth and 25°C for 2-4 days for fungi growth. Colonies were counted in order to obtain the total viable microbial load, discrete colonies were purified by sub-culturing into new prepared agar media and growth was observed under the microscope and then characterized using standard method.

2.3.2. Enumeration of Microbial Colonies

Colony counting was carried out visually by counting the number of visible colonies that appeared on the plates. Calculation of colony forming unit (CFU) per ml for the bacteria and the spore-forming unit (SFU) per ml for the fungi was based on the formula:

$$\text{CFU/ML or SFU/ML} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Ml of sample suspension (Fawole and Oso, 2007)}}$$

Concentration of analyte (mg/g)=(Peak Area of sample ×Standard Concentration)/(Peak Area of the Standard)

2.3.3. Gram's Staining

This was carried out to establish the Gram reaction of the isolates. A smear of each colonies were made on glass slides, crystal violet stain was added to the smear for 30-60 seconds, after which the stain was washed off with sterile water, then iodine was added to the smear for another 30-60 seconds to fix the stain and washed off with sterile water. Alcohol was added to the smear for 60 seconds to decolorize the stain; safranin red was finally added to the smear as a counter stain. The glass slides were mounted on microscope and observed under oil immersion objectives lens (x100). A purple colour indicated the presence of Gram-positive microorganism while red/pink colour indicates the presence of a Gram-negative microorganism (Fawole and Oso, 2007).

2.4. Biochemical Characteristics

2.4.1. Determination of the Presence of Catalase

The isolates were tested for the presence of enzyme catalase by placing a small amount of 24 hours old bacterial culture on a clean glass slide using a sterile inoculating loop, few drops of 3% hydrogen peroxide was added to the smear, presence of bubbles indicates liberation of oxygen that is, the presence of enzyme catalase which breaks down H_2O2 to H_2O and O_2 (Fawole and Oso, 2007).

2.4.2. Determination of the Presence of Citrate

The isolates were inoculated into Simmons citrate agar slants and incubated at room temperature for 24 hours. Organisms that are able to utilize citrate as their source will be indicated by a colour change of the agar from green to deep blue, while microorganisms that cannot utilize citrate will be indicated by no colour change (Cheesbrough, 2006; Fawole and Oso, 2007).

2.4.3. Determination of Motility

This was carried out to establish the ability of the isolates to move due to the presence of a structure called flagella. It was carried out by inoculating nutrient agar medium (with reduced percentage of agar component of the medium) with a straight wire, making a single stab down the centre of the tube to about half the depth of the medium. The tubes were then incubated at 37°C. Non-motile bacteria will give growths that are confined to the stab line, while motile bacteria give diffuse growth that spread throughout the medium (Fawole and Oso, 2007).

2.4.4. Determination of Starch Hydrolysis

About 1.4 gramme of nutrient agar and 1.0 gramme soluble starch were prepared in 100 ml distilled water and sterilized at 121°C for 15 minutes, allowed to cool, poured on sterile plates, and inoculated by single streak of the isolate across the surface of the media. It was incubated at 37°C for 24 hours. The control and experimented plates were incubated for 5 days. Hydrolysis of starch was determined by flooding the plates with Lugol's iodine. The clear zone around the colony shows positive, blue-black colour shows negative, while reddish brown zones around the colony show partial result. Un-inoculated tubes serve as control for the test (Fawole and Oso, 2007).

2.4.5. Determination of the Presence of Methyl Red

A 9 ml buffered (phosphate) glucose broth (1 g glucose, 0.5% KH_2PO_4, 0.5% peptone, and 100 ml distilled water) were dispensed aseptically into clean test tubes and covered with absorbent cotton wool and aluminium foil, sterilized at 121°C for 15 minutes. After cooling, it was inoculated with bacterial isolate and incubated at 37°C for 48 hours. About five (5) drops of methyl red reagent was added to each test tubes, development of bright red colour indicate negative result. Un-inoculated tubes serve as control for the test (Fawole and Oso, 2007).

2.4.6. Determination of Sugar Fermentation

Some microorganisms have the ability to metabolize varieties of sugar as carbon source produced by the organisms. Fermentation occurs when carbohydrates are used in the total or partial absence of oxygen. Glucose, galactose, sucrose, lactose, and maltose are used for this fermentation test. A 5 ml each of the sugar solution was dispensed into different tubes with Durham's tube were inverted into each test tube. The mouths of the tubes were plugged with cotton wool and labelled appropriately; it was then sterilized in an autoclave for 15 minutes at 121°C. The tubes were then allowed to cool down. The bacterial isolates were inoculated aseptically into the sugar solutions in the tubes and incubated at 37°C for 72 hours. The change of colour from red to yellow indicates acid production, which implies the utilization of the sugar by the microorganism, and appearance of bubble in the Durham's tube indicates gas production, but if otherwise, acid or gas is not produced (Adetunji et al., 2012; Fawole and Oso, 2007).

2.5. Determination of Thiobarbituric Acid (TBA)

Weighed accurately 5 g of the sample with 50 ml of distilled water for 2 minutes and wash into distillation flask with 47.5 ml of water. Add 25 ml of 4MHCl to bring the pH to 1.5 followed by an antifoam preparation. Heat the flask by means of an electric mantle so that 50 ml distillate is collected in 10 minutes from the time boiling commences. Pipette 5 ml distillate into a conical flask, add 5 ml TBA reagent, shake, and heat in a boiling water bath for 35 minutes and read the absorbance at 538 nm against reagent blank (Asakawa and Matsushita, 1979).

2.6. Statistical Analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS), version 26.0 and the difference between mean were considered significant at P value < 0.05 (95 % confidence interval) .

2.7. Determination of Vitamin

2.7.1. Vitamin A Protocol

The quantity of sample weighed must not contain more than 1 g fat and at least 240 unit of vitamin A with 30 ml absolute alcohol and 3 ml of 5% potassium hydroxide boil gently under reflux for 30 minutes in a stream of oxygen free nitrogen. Cool rapidly, add 30ml water, transfer to separator, wash in with 3 X 50 ml ether, and extract the vitamin A by shaking for 1 minute. After complete separation, discard the lower.

2.7.2. Determination of Vitamin C

The vitamin C content was determined using the ascorbic acid as the reference compound.200 µl of the extract was pipetted and mixed with 300 µl of 13.3% of TCA and 75 µl of DNPH. The mixture was incubated at 37°C for 3 hrs and 500 µl of 65% H_2SO_4 was added. Add 500 ul of water. The absorbance was read at 520 nm (Benderitter *et al.,* 1998). Blank is made up of all reagents without the sample.

2.7.3. Determination of Vitamin B1 (Thiamin)

5 g of the sample was homogenized with 50 ml ethanoic sodium hydroxide. It was filtered into a 100 ml conical flask. 10 ml of the filtrate was pipette and the colour was develop by addition of 10 ml of 1%potassium dichromate and read the absorbance at 360 nm. A blank solution is also prepared. (Okwu and Josiah, 2006).

2.7.4. Determination of Vitamin B2 (Riboflavin)

5 g of the sample was extracted with 100 ml of 50% ethanol and shaken for one hour. This was filtered into 100 ml flask 10 ml of the extract was pipette into 50 ml volumetric flask. 10 ml of 5% potassium permanganate and 10 ml of 30% H_2O_2 was added and allowed to stand over a hot water bath for 30 minutes. 2 ml of 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance measured at 510 nm using spectrophotometer.

2.7.5. Estimation of Ascorbic Acid

The amount of ascorbic acid present in the leaves, flowers and fruit pulp of *Couroupita guianensis* was estimated by the method of (Roe and Keuther 1943).

Principle

Ascorbate is converted to dehydroascorbate by treatment with activated charcoal or bromine. Dehydroascorbic acid then reacts with 2,4- dinitrophenyl hydrazine to form osazones, which dissolves in sulphuric acid to give an orange colour solution. The coloured product can be measured spectrophotometrically at 540 nm.

Procedure

The samples of 1 g were taken and homogenized with 4% TCA to extract the ascorbate and the final volume was made up to 10 ml with 4% TCA. The supernatant obtained after centrifugation at 2000 rpm for 10 minutes was treated with a pinch of activated charcoal, shaken well, and kept for 10 minutes. Centrifugation was repeated once again to remove the charcoal residue. The volumes of the clear supernatants obtained were noted. Two different aliquots of the supernatant were taken for the assay (0.5 ml and 1.0 ml). The assay volumes were made up to 2.0 ml with 4% TCA. A range of 0.2 to 1.0ml of the working standard solution containing 20-100 µg of ascorbate, respectively, were pipetted into clean, dry test tubes, the volumes of which were also made up to 2.0 ml with 4% TCA. DNPH reagent (0.5 ml) was added to all the tubes, followed by two drops of 10% thiourea solution. The osazones formed after incubation at 37°C for 3 hours were dissolved in 2.5 ml of 85% H_2SO_4, in cold conditions, to avoid an appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of H_2SO_4. After incubation for 30 minutes at room temperature, the samples were read at 540 nm, and the levels of ascorbic acid in the samples were determined using the standard graph constructed on an electronic calculator set to the linear regression mode and expressed as mg ascorbate/g sample.

2.8. Proximate Analysis

Total ash content was determined by incineration of each fermented sample at 600°C (AOAC, 2012). About 1 g of the sample was weighed into a pre-weighed crucible and incinerated in a furnace at 600ºC. The crucible was removed and cooled in a desiccator and weighed. Ash content was calculated according to the follow formula:
Where; Aw = Ash weight and Sw = Sample weight.

2.8.1. Determination of Crude Fat

Solvent extraction method was used according to (AOAC, 2012). About 2 g from each fermented sample was weighed into a labelled thimble and 250 ml boiling flasks. The thimble was plugged with cotton wool and 300 ml of petroleum ether was added to the sample in the boiling flasks. This was reflux with Soxhlet extractor apparatus to carryout extraction for 6 hours. The petroleum ether was emptied into a container and percentage (%) fat calculated by the formula:
Where; W1= weight of fat, W2= weight of sample

2.8.2. Determination of Crude Protein

According to AOAC (2012), 10 g from each fermented sample was transferred into 50 ml Kjeldahal flasks. About 2ml of distilled water was added to the flasks and allowed to stand for 30min. Thereafter, 0.02g powdered pumice, 1.33 g K_2SO_4 catalyst mixture, and 1.5 g concentrated H_2SO_4 were added to it. This was heated on a digestion rack until frothing stopped. Heating was increased to gentle boiling to condense H_2SO_4 to about one third. The isolated particle was washed with 30% H_2O_2 solution before boiling again for 1 hour and allowed to cool. 10 ml of deionized water was added slowly with swirling. About 2 ml aliquots of each diluted solution were measured, and total crude protein was determined using a spectrophotometer.

2.8.3. Determination of Moisture Content

According to AOAC (2012). about 10 g from each fermented samples substrate were weighed and oven dried at 131°C for 2 hours. The loss in weight, using the formula below, gave the moisture content of the original sample.
Where M= original mass of substrates in grams, m= final mass of dried sample in gram.

2.9. Statistical Analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS), version 26.0 and the difference between mean were considered significant at P value < 0.05 (95%confidenceinterval) .

3. RESULTS AND DISCUSSION

3.1. Total Viable Plate Count of Microorganism Present in Soursop Before and After Storage in Room and Refrigerated Conditions

In this study, the effect of different storage condition on the microbiological and nutritional quality of Soursop juice were evaluated. It was observed that the bacteria count (1.20 cfu/ml) and coliform count (2.17 cfu/ml) of soursop juice were low during the first seven days and there was a subsequent increase in bacteria count (24.10 cfu/ml) and coliform count (26.13 cfu/ml) after seven days storage in room temperature. The results also revealed that in refrigerated temperature the bacteria load was too numerous to count, but coliform count was low (1.62 cfu/ml). The variation in microbial loads of fruits in the study areas could be due to contamination by microorganism from soil, irrigation water, the environment during transportation, washing/rinsing water, or handling by processors, or may be part of the natural flora of the fruits, as reported by other authors (Ofor *et al.,* 2009: Singh *et al.,* 2013).

In addition, the different species of bacteria isolated from the fruit samples could be due to poor storage conditions, as well as handling and processing of the fruits (Eni *et al.,* 2010; Singh *et al.,* 2013). Similar species of bacteria were also isolated by many researchers in an attempt to determine or identify microbial populations present in fruits or responsible for their Spoilage (Uzeh *et al.,* 2009; Bukar *et al.,* 2010). It was observed from the results that the growth of Yeast/mold was favoured. Yeast growth in soursop was favoured by the presence of sugar and acid pH. Fruit juices are readily fermented by yeast, while their acid pH discourages most bacterial growth. Similar trends in reduction were noted by (Gow-Chin and Hsin-Tang, 1996; Sadler *et al.,* 1992). The results have therefore shown that the microbiological quality of soursop juice was affected by storage temperature.

3.2. Cultural and Biochemical Characteristics of Organisms Present in Soursop Before and After in Room and Refrigerated Conditions

The biochemical characterization of the isolated organisms (Table 2) shows that all the three isolated organisms were gram positive (*Staphylococcus arlettae, Bacillus pumilus and Staphylococcus xyloxus*) before storage. The biochemical characterization of the isolated organisms (Table 3) shows that after seven days storage in room temperature, the three of the isolated organism were Gram positive (*Staphylococcus xylosus, Staphylococcus arlettae and Bacillus tequilensis*). Likewise, storage in refrigerator, three were gram positive (*Staphylococcus arlettae and Bacillus pumilus*).

Cultural and biochemical characteristics of organisms present in Soursop juice is presented in Table 2 and 3 revealed a difference in the cultural identification of *Bacillus pumilus and Paenibacillus thailander*, isolated from Soursop juice before seven days storage. The presence of these micro-organisms in soursop indicates contamination. Moreover, species of Staphylococci and other pathogenic and opportunistic species detected in the fruit samples could lead to food borne illnesses since the fruits are usually consumed raw (Bukar *et al.,* 2010; Eni *et al.,* 2010). Staphylococcus *xylosus, Staphylococcus arlettae,* and *Staphylococcus tequilensis* were isolated after seven days storage in room and refrigerated temperature. It has been reported that moisture impacts effectively on microbial growth, thus the rotten fruits with more moisture levels have more number of microorganisms. This work indicated that bacteria and fungi are the most predominant organisms of this fruit.

This is in support with the research conducted by Ajiboye *et al.* (2014). This variation could be as a result of the differences in the sources of sample collection.

3.3. The Result of the Proximate Analysis of Soursop Juice

The proximate analysis carried out on soursop juice is presented in Table 4. The statistical analysis results showed that there is a significant difference ($p < 0.05$) in the percentage quantity of moisture, ash, protein, fat, fibre, and carbohydrate in the samples of the Soursop juice. Evidence is clear from the result of the proximate analysis that fresh Soursop before seven days storage have high moisture content, which make it sources of fluid to the body when consumed (Olawale *et al.,* 2023). High moisture content is a characteristic feature of juices and fresh fruits are known to possess more water, especially when juiced. This is in consonance to United States Department of Agriculture (USDA) document on water composition of food. Henry-Unaeze and Daniel (2021) carried out a similar analysis on fresh Soursop samples, which yielded high moisture content ranging from 85.05 to 86.45%. The lower moisture content of juice blends could mean that they will have a relatively longer shelf life than juices with higher moisture content, as high moisture encourages the growth of microorganisms and reduces storage life (Henry-Unaeze and Daniel). However, there was a significant increase in the soursop moisture content both in room and refrigerated temperature (70.12% and 74.45%) this shows that the juice retained it moisture content value even after storage in different conditions.

In addition, the percentage of ash both before storage and after seven days storage in room and refrigerated temperature is higher in this study than the study on soursop juice conducted by Abbas *et al.* (2015). Ash content of any food is directly related to its mineral content (Henry-Unaeze and Daniel, 2021). This indicates that the soursop blend contributes to the mineral content of the juice. The fat content in this study is higher both before storage and after seven days storage in room and refrigerated temperature than the research conducted by Henry-Unaeze and Daniel (2021). The soursop juice in this study could therefore be suitable for people who are interested in weight management and in the prevention of diseases associated with a high dietary fat intake or consumption (Olawale et al., 2023). All the same, the general low-fat value of fruit juices is advantageous to good health. Moreover, the fibre content in this present study is lower than the study conducted by Degnon *et al.* (2013).
Crude fibres are also present in the soursop fruit. Although crude fibre does not contribute to nutrients or energy, it is a source of dietary fibre. According to Wardlaw and Kessel (2002), fibre aids in the alleviation of flatulence problems, hence tiger nuts fibre could be explored in formulating diets for treating indigestion, constipation, and non-communicable diseases such as colon cancer, diverticulosis, coronary heart disease, and obesity. 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). The low protein value of the blends makes the juice blends ideal for low protein diet. Thus, soursop fruits are a potentially good source of proteins, which should be exploited to determine if they are commercially viable. The carbohydrate content in this study is high at before storage (24.08%) with a significant reduction in room and refrigerated temperature after seven days storage. The blends carbohydrate value is of nutritional benefit as the juices could be used to temporarily sustain hunger. The proximate analysis of soursop shows that it can be a good substitute fruit juices order to meet their nutritional needs, especially for protein, fat, and fibre content.

3.4. The Result of Vital Vitamin in Soursop Juice

The results of the analysis carried out on soursop juice revealed that there is difference ($p<0.05$) in the quantity of four vitamin tested for in the samples of soursop juice. Vitamins composition of fresh Soursop juice samples after undergoing analysis was highest in Vitamin (674.26±1.89b) followed by Vitamin C (71.01±0.93c), then Vitamin B1 (1.22±0.00a) to Vitamin B2 (0.69±0.00a) as seen in Table 5

Result of the vitamin composition in soursop after seven days storage shows that Vitamin A (315.16±48.11a) and Vitamin C (39.52±0.06b) reduced after seven days storage in room temperature but Vitamin A (551.15±1.89b) increased in refrigerated temperature while Vitamin C (36.98±0.06a) composition remain reduced in refrigerated temperature. Both Vitamin B1 (2.68±0.00c) and B2 (2.50±0.00c) was higher in value in room temperature after seven days storage, with a subsequent reduction in refrigerated temperature after seven days storage, both in Vitamin B1 (2.11±0.00b) and B2 (2.29±0.00b) as seen in Table 5

The vital vitamin present in Soursop shows varying differences as presented in table 5. In this study, Vitamin A content was the highest. High Vitamin A improves vision, and when Vitamin A levels are inadequate, the lack of rhodopsin makes it difficult to see in dim light (Gräslund et al., 2008). Vitamin B1 is also important as other vitamins in soursop. Thiamine is a co-catalyst in sugar digestion and is necessary to the function of the heart, nerves, and muscles (Wiley and Gupta, 2019). It is very important in human diet. Analysis carried out on soursop in this study shows it contains higher vitamin B2 content than that conducted by Abara and Ugwu (2017) (0.24). Deficiency of riboflavin can lead to Ariboflavinosis, which is characterized by cheilosis (textured desquamation of the skin around the mouth), glossitis (sparkly red, and sore tongue), soreness of the lips, eye disturbances and photophobia (light sensibility), oily skin the nose, scrotal dermatitis (Henriques *et al.,* 2010). However, presence of vitamin C in high amount in soursop juice prevent coronary disease and cancer (Wardlaw and Kessel, 2002). Vitamin C have been shown to decrease oxidative stress and atherosclerotic processes, with potential roles in the prevention of lipid peroxidation and atherosclerosis. This study revealed that soursop is very important as an essential source of vitamin in supplementary diet.

Table 1. Total viable plate count of microorganism present in Soursop before and after storage in room and refrigerated conditions.

	TVBC($\times10^2$ CFU/ml)	**TVCC($\times10^2$ CFU/ml)**	**TVY/MV($\times10^2$ SFU/ml)**
SS (B7)	1.20±0.06^a	2.17±0.09^b	19.62±0.01^b
SS (A7) R	24.10±0.06^b	26.13±0.09^c	4.10±0.06^a
SS (A7) RT	TNTC	1.62±0.01^a	TNTC

Values are presented as mean ± SE of triplicates. Values in the same column carrying the same superscript are not significantly different at $p < 0.05$ according to Duncan's New Multiple Range Test.

Key: TVBC- Total viable bacteria count, TVCC- Total viable coliform count, TVY/MV-Total viable yeast and mold count, A7- After 7 days of storage, B7 – Before 7 days of storage, SS – Soursop, R-Room, RT-Refrigerated

Table 2. Cultural and biochemical characteristics of organisms present in Soursop before storage in room and refrigerated conditions.

Sample(B7)	Gram stain	Shape	Gas	H_2S	Glucose	Rhamnose	Sucrose	Lactose	Methyl Red	V.P	Casein	Lipase	Ribose	Arabinose	Mannitol	Glycerol	Galactose	Sorbitol	Citrate	starch	Motility	Indole	Maltose	Raffinos+	Organism suspected.
ISOLATE 1	+	Rod	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	-	+	+	*Bacillus pumilus* Similarity: 87.3% Probability: 5% Matrix integrity: 100%
ISOLATE 2	+	Rod	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+	-	+	-	+	+	*Bacillus pumilus* Similarity: 87.3% Probability: 1.2% Matrix integrity: 100%
ISOLATE 3	+	Rod	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	-	+	-	+	+	*Paenibacillus thailander* Similarity: 89.1% Probability: 20.4% Matrix integrity: 100%

Table 3. Cultural and biochemical characteristics of organisms present in Soursop after storage in room and refrigerated conditions.

Sample(A7)	Gram stain	Shape	Gas	H_2S	Glucose	Rhamnose	Sucrose	Lactose	Methyl Red	V.P	Casein	Lipase	Ribose	Arabinose	Mannitol	Glycerol	Galactose	Sorbitol	Citrate	starch	Motility	Indole	Maltose	Raffinos+	Organism suspected.
ISOLATE 1 RFT	+	Rod	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	*Bacillus pumilus* Similarity: 87.3% Probability: 5% Matrix integrity: 100%
ISOLATE 1 RMT	+	Cocci	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+	+	-	+	-	*Staphylococcus xylosus* Similarity: 89.3% Probability: 1.2% Matrix integrity: 100%
ISOLATE 2 RFT	+	Cocci	-	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	*Staphylococcus arlettae* Similarity: 87.7% Probability: 2% Matrix integrity: 100%
ISOLATE 2 RMT	+	Cocci	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	*Staphylococcus arlettae* Similarity: 98.3% Probability: 11.5% Matrix integrity: 100%
ISOLATE 3 RFT	+	Cocci	+	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	*Staphylococcus arlettae* Similarity: 90.7% Probability: 0.1% Matrix integrity: 100%
ISOLATE 3 RMT	+	Rod	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	*Bacillus tequilensis* Similarity: 94.1% Probability: 31% Matrix integrity: 100%

- RFT- Refrigerated Temperature
- RMT- Room Temperature

Table 4. The Result of the Proximate Analysis of soursop Juice.

Treatments	Moisture%	Ash%	Fat%	Fibre%	Protein%	Carbohydrate%
SS Day 1	63.10±0.03[a]	8.27±0.04[c]	1.07±0.04[c]	0.12±0.00[a]	3.37±0.01[c]	24.08±0.10[c]
SS A7 (R)	70.12±0.01[b]	5.54±0.04[a]	0.80±0.05[b]	0.15±0.00[b]	2.53±0.02[a]	20.67±0.20[b]
SS A7(RT)	74.45±0.02[c]	6.75±0.01[b]	0.63±0.01[a]	0.17±0.00[c]	2.87±0.05[b]	15.14±0.05[a]

Values are presented as mean ± SE of duplicates. Values in the same column carrying the different superscripts are significantly different at $p < 0.05$ according to Duncan's New Multiple Range Test.

Table 5. The Result of Vital Vitamin in Soursop Juice.

Treatments	Vit A	Vit B1	Vit B2	Vit C
SS	674.26±1.89[b]	1.22±0.00[a]	0.69±0.00[a]	71.01±0.93[c]
SS (R)	315.16±48.11[a]	2.68±0.00[c]	2.50±0.00[c]	39.52±0.06[b]
SS (RT)	551.15±1.89[b]	2.11±0.00[b]	2.29±0.00[b]	36.98±0.06[a]

Values are presented as mean ± SE of duplicates. Values in the same column carrying the same superscript are not significantly different at $p < 0.05$ according to Duncan's New Multiple Range Test.

4. CONCLUSIONS

Soursop as a tropical fruit showed good potential for use in the formulation of a functional drink. It also displayed potential for incorporating unique raw materials such as honey (a highly medicinal and nutritious food) and milk (to improve its mouthfeel, taste and to impart some creaminess) for value addition purposes. The increased vitamin content and significant nutritional properties may increase the willingness of consumers to buy and consume the drink, thereby promoting its utilization and industrial processing as well as improved cardiovascular health of the consumers. Storage temperature greatly affects the microbiological stability and hence the quality of the soursop juice. It is recommended that studies should be conducted on the effect of packaging and use of chemical preservatives on the storage stability of soursop juice. However, as part of recommendation studies should be conducted to reduce the susceptibility of the fruit to microbial spoilage and to ensure its effectiveness in different applications to preserve the shelf life.

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