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# Production and Properties of Probioticic Soursop Juice Using *Pediococcus pentosaceus* LBF2 as Starter

**Stephanie Clara Akpeji[1], Bukola Christianah Adebayo-Tayo[1*], Jadesola Fawzhia Sanusi[2] and Solomon Omoniyi Alao[1]**

*[1]Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria.*
*[2]Department of Biological Sciences, Crescent University, Abeokuta, Ogun State, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author SCA carried out the laboratory work under the supervision of author BCAT and wrote the first draft of the manuscript. Author BCAT wrote the protocol and supervised the work. Authors JFS and SOA managed the literature search and analyses of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Production of probiotic soursop juice and determination of properties of the juice samples for 3 weeks of storage at 4℃.
**Study Design:** To determine the properties of soursop juice produced using *Pediococcus pentosaceus* LBF2 as starter culture.
**Place and Duration of Study:** Department of Microbiology, University of Ibadan, Ibadan, Oyo state, Nigeria between July to December 2016.
**Methodology:** Production of soursop juice using *Pediococcus pentosaceus* LBF2 as probiotic starter and determination of viability of the starter, physicochemical, nutritional and organoleptic properties of the samples kept at 4℃ for 3 weeks wa s determined. The soursop juice with probiotic starter was labeled Psop and the soursop juice without the probiotic starter was labeled Pcont.

**Corresponding author: E-mail: bukola.tayo@gmail.com;*

**Results:** The probiotic strain was viable in the samples during storage ($1.57\times10^7$ CFU/mL – $7.9\times10^7$CFU/mL) and the highest viability ($1.85\times10^7$CFU/mL) was recorded at 2 weeks of storage. The lactic acid content of the probioticated samples ranged from 1621.44 - 2450.176 mg/l during storage. There was a general reduction in the pH of the probioticated samples. The Total Soluble Solid of the Psop and Pcont samples ranged from 15 – 6.0 and 13.5 - 8.0.

The samples were acceptable and strongly liked in terms of sensory attributes in the 1st and 2nd week of storage. The Pcont samples were extremely disliked in the 4th week of storage. The crude protein, crude fat, crude fiber of the samples ranged from $0.58^b$ - $0.65^a$%, $0.04^c$ – $0.07^a$% and $0.48^c$- $1.91^a$%. The highest crude protein was recorded at week 2 and 3 of storage. There was a general reduction in the crude fat, crude fiber and carbohydrate content during storage.

**Conclusion:** In conclusion the probioticated samples had a longer shelf-life, contain viable probiotic candidate, strongly acceptable with good nutritional composition which can confer a strong health beneficial effect on the consumer.

*Keywords: Probiotication; soursop juice; storage; physicochemical; proximate; sensory.*

## 1. INTRODUCTION

Probiotic foods are products to which probiotics has been added and they are group of functional foods with growing market shares and large commercial interest [1]. Probiotics are live microorganisms which, when consumed in large quantities, confer health benefits [2] to the consumer. Recently the popularity of the potential health benefits of fermented foods as functional foods has increased. Research are been made into the physiology and functional properties of the microbes present in fermented food which are equally used as function foods [3]. Probiotics are active against colon infections, gastro intestinal disorders and in the maintenance of healthy gut microflora [4]. Enriching and making foods with probiotics would not only improve public health but also the diversity in food choices. *Pediococcus* are gram positive cocci, acid tolerant and possess a strictly fermentative metabolism with lactic acid as the major metabolic product [5,6]. *Pediococcus pentosaceus* can be isolated from a variety of plant materials and bacteria ripened cheese.

Fruit juices could serve as a good medium for cultivating probiotics because they are rich in nutrients and do not contain starter cultures that compete for nutrients with probiotics [7]. Most of the tropical fruits are important source of antioxidants, vitamins, dietary fibres and minerals; and form a very healthy part of our diet [8]. They contain oxygen demanding ingredients such as ascorbic acid, thus promoting anaerobic conditions. Fruit juices contain high amounts of sugars which could encourage probiotic growth and they lack allergens, as in case of dairy products, that might cause allergy in certain segments of the population [9].

Soursop, also known as guanabana (*Annona muricata* ), belong to the family *Annonaceae* and is an exotic, nutritive, pleasant, sub-acid, aromatic and juicy fruit [10-11]. Soursop fruits are good source of vitamins, dietary fiber and minerals and provide flavor, aroma and texture to the pleasures of eating food but also are claimed to have anticancer and antioxidant capabilities [12]. Soursop is commonly found in Southern part of Nigeria. They are mostly consumed fresh and as such can become a potential source of raw material for puree, juice, jam, jelly, powder fruits bars and flakes. As a result of the current trend of nutritious fast food, juice dehydration for use in instantaneous beverages has become an interesting alternative to artificial juices available in the market [13]. Sourpsop can be used as medicine [14] and as carrier for probiotics.

The correlation between diet and health is increasing day by day therefore, healing an illness through particular food consumption to restore natural defense with fewer side effects than medicine is always appealing to all age groups [15]. Thus research and development on functional food components like probiotics, phytochemicals, and omega fatty acids is presently an important focus of the food industry [16]. In addition to very high-priced health care and medicines, the desire for better quality of life encourages novel functional foods consumption with multiple health benefits apart from basic nutrition [17].

This research work aimed at production of sour sop juice using *Pediococcus pentosaceus* as probiotic starter and determination of the viability and physicochemical properties of the juice samples during storage.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Laboratory Preparation of Soursop Juice

The fresh fully ripe soursop fruit was purchased from Ojo market in Ibadan, Oyo State Nigeria. The washed fruit samples were hand peeled, decored, deseeded and the pulp blended using an electric blender. Sterile distilled water was added in the ratio of 1:2 (w/v, pulp/water) to facilitate the blending process. The pulp was filtered using a sterile muslin cloth. The juice was pasteurized at a temperature of 85℃ for 30 minutes. After cooling, samples were stored at 4℃ before they were used for further analysis.

#### 2.1.1 Culture collection and inoculums preparation

Probiotic *Pediococcus pentosaceus* LBF2 was obtained from the culture collection of our previous work in the Department of Microbiology, University of Ibadan. The stock cultures were maintained on MRS agar and stored at 4℃. 0.5 Mac Farland standard suspension with turbidity of $1.5\times10^8$ cfu/mL [18] was used to standardize the inoculums. The seed culture was grown in a 250 mL flask containing 50 mL of sterile MRS broth. Cells were harvested by centrifugation (10,000 g) for ten minutes and washed twice by sterile 0.1% peptone buffer [19]. The cultures were diluted with sterile distilled water and the optical density of bacterial suspension was measured using spectrophotometer at 625 nm. The optical density of bacterial suspension was compared with the optical density of 0.5 Mac Farland containing 1.5 $x10^8$ cfu/mL [18]. Serial dilution was done to obtain dilution $10^7$ cfu/mL of the probiotic *Pediococcus pentosaceus* LBF2.

### 2.2 Production of the Probioticated Soursop Juice Samples

Probiotication of pasteurized juice samples was done by inoculating 100 mL of the pasteurized soursop juice sample with 1% (v/V) equivalent to 10 mL of the probiotic *Pediococcus pentosaceus* LBF2 starter (0.5 Mac Farland standard containing 1.5 $x10^8$ cfu/mL). The inoculated samples were labeled Psop and the Un-inoculated juice samples (Pcont) serves as control. The samples were incubated at 37℃ for 72 hrs and stored at 4℃ for four weeks. Weekly analysis such as the lactic acid, pH, Total Soluble Solid, color and vitamin C was determined. The flow diagram for the production of soursop juice is shown in Fig. 1.

Soursop
↓
Washing
↓
Peeling
↓
Cutting
↓
Deseeding
↓
Extraction of soursop juice
↓
Pasteurization
↓
Probiotication of pasteurized soursop juice
↓
Fermentation (72 h)
↓
Storage at 4℃
↓
Weekly analysis of the stored probiotic soursop juice samples

**Fig. 1. Flow diagram for the preparation of probioticated soursop juice**

### 2.3 Determination of the Viability of the Probiotic Strain Inside the Stored Soursop Juice Samples

The viability of the *Pediococcus pentosaceus* LBF2 in the Psop and Pcont soursop juice samples was determined using a pour plate technique [20]. The samples were pour plated at weekly intervals. 1 mL of the sample was inoculated on MRS agar plates and incubated at 37℃ for 48 hrs. Distinct colonies were counted and the numbers were multiply by the reciprocal of the dilution factor and expressed as colony forming the unit (CFU).

### 2.4 Quantitative Estimation of Lactic Acid, pH, Colour and Total Soluble Solid of the Stored Samples

The production of lactic acid by *Pediococcus pentosaceus* LBF2 inside the stored samples was determined by titrating 10 mL of the homogenized sample against 0.25 mol l-1 NaOH using 1 mL of phenolphthalein indicator (0.5% in 50% alcohol). Each milliliter of 1 N NaOH is equivalent to 90.08 mg of lactic acid [21].

The pH of the probioticated soursop juice samples stored at 4°C for 1- 4 weeks was determined using a pH meter (Titumum U9N model).

Colour assessment of the probioticated soursop juice sample stored at 4°C for 1- 4 weeks was done by using the colour meter. The colour parameters (Hunter L*, a*, and b* value) were determined for each sample using a spectrophotometer – colorimeter (CM-2500D Minolta, Japan) according to the method of Maskan [22].

Total soluble solids (TSS) of the stored probioticated soursop juice samples weeks were determined using a hand refractometer (Erma, Japan) in terms of °Bx (°Brix) [22].

### 2.5 Sensory Evaluation of Stored Probioticated Soursop Juice Samples

Coded samples of the probioticated soursop juice samples were served to 10 trained panelists. The panelist was asked to rate the sample based on taste, aroma, colour and appearance. Triplicate determinations were made per sample. The ratings were presented on a 7 point hedonic scale ranging from 1 - extremely like, 7 - extremely dislike. Results obtained were subjected to analysis of variance using one-way ANOVA, the difference between means was separated using Duncan's Multiple Ranged Test [23].

### 2.6 Determination of Proximate Composition of the Soursop Juice Samples

The proximate composition (Crude protein, crude fat, crude fiber, ash and carbohydrate content) of the stored probioticated juice samples was determined according to the method of AOAC [24]. The determination was done in duplicate.

## 3. RESULTS AND DISCUSSION

The properties of the probioticated soursop juice stored at different time interval was evaluated.

Viability of probiotic *Pediococcus pentosaceus* LBF2 in the soursop juice sample is shown in Fig. 2. The probiotic strain was viable in the samples during 4 weeks of storage. The viability ranged from log 5.897 - 7.267 CFU/mL and the highest viability was recorded at 2 weeks of storage.

The viable cell counts of the *Pediococcus pentosaceus* LBF2 in the soursop juice were higher than $10^6$ CFU/mL after 4 weeks of cold storage at 4°C. It was observed that *Pediococcus pentosaceus* LBF2 had the high growth during fermentation and the maximum viable cell counts during 2nd week of cold storage. High cell viability recorded in this work support the findings of Anita et al. [25] that higher viable cell numbers are obtained during immobilized cell fermentation than during free cell fermentation. However, it is important to have a significant number of viable LAB in the product for maximum health benefits [26].

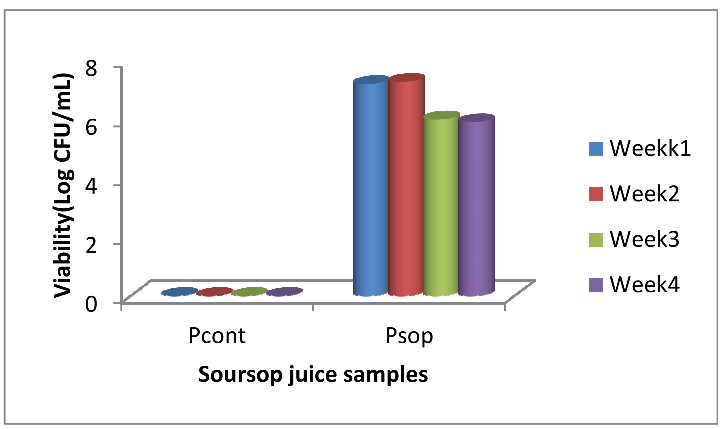


**Fig. 2. Viability (log CFU/mL) of probiotic *Pediococcus pentosaceus* LBF2 in soursop juice sample**

Reduction in population during the 3rd and 4th weeks of cold storage at 4℃ may be attributed to pH reduction in the fermentation medium, accumulation of organic acid as a consequent of bacteria growth and fermentation [9]. This observation is in agreement with the report of Yanez et al. [27] who reported that some conditions such as increased acidity resulting from the fermentation process can reduce the survival and viability of probiotic bacteria.

Consequently, fermented soursop juice could be considered as a probiotic beverage without any nutrient supplementation. This observation is in support of the report of Anita et al. [25] on probiotication of fruit juices by *Lactobacillus acidophilus.*

The viability and activity of probiotic bacteria during preparation and storage is very important for their industrial preparation. Shah [28] reported that the viability of probiotic organisms is dependent on many factors, such as the level of oxygen in products, oxygen permeation of the package, fermentation time and storage temperature.

## 3.1 Lactic Acid Production, pH and Total Soluble Solid (TSS) of the Probioticated Soursop Juice

The lactic acid content in the Psop and Pcont juice samples is shown in Fig. 3. It ranged from 648.576 – 1460.197 mg/l and 1621.44 – 2450.176 mg/l for Pcont and Psop samples. Reduction in lactic acid was observed in the Pcont juice samples during storage. The lowest was recorded at 4th week of storage. An increase in lactic acid was observed in the Psop juice sample during storage. The highest lactic acid was produced in the 2nd and 3rd week of storage.

The pH of the Psop and Pcont soursop juice samples is shown in Fig. 4. There was variation in the pH of the Psop juice samples. The pH ranged from 2.7 - 3.84 and 4.2 – 4.32. The lowest pH was obtained in the 3rd week of storage and there was no reduction in the pH of the Pcont juice sample during the storage time. Variation in pH may be due to metabolic activities in the fermentation medium which may lead to the breaking down of the fruit sugar to produce organic acids.

The TSS of the Psop and Pcont juice samples is shown in Fig. 5. It ranged from 6.0 – 15.0 and 8.0 – 13.5, the least was recorded at week 4 of storage. There was a general reduction in the TSS of the samples during storage. There were significant differences ($P \leq 0.05$) in the TSS of the Psop samples while there was no significant difference ($P > 0.05$) in the TSS of the Pcont juice samples.

Decrease in pH in the beginning of fermentation had a great importance for the quality of the end product [29]. Increase in acidity during probiotication minimizes the influence of spoilage bacteria. The results of this study showed that, the growth of *Pediococcus pentosaceus* LBF2 in soursop juice lead to pH reduction and increase in lactic acid. The main reason of this case has been attributed to the sugars consumption and production of organic acid by lactic acid cultures [30].

The physicochemical properties of probioticated fruit juices were studied at different storage intervals (weeks). The fruit juice mostly contains high concentration of acids with good organoleptic properties. These are also a good source of antioxidants, vitamins and minerals. Fruits in the form of fresh fruit juices are included in regular diet. Fruits and fruit juice based drinks are important components of human diet, people choose fruit juices as a drink for many reasons, including relieving thirst, refreshment and nutrition benefit [9]. The reduction in the carbohydrate may be due to utilization of the same for the growth of the bacteria. Decrease in TSS at the end of the fermentation and storage was due to the consumption of sugar in the probioticated soursop juices [31]. At the end of the fourth week of storage of the Soursop juice at 4℃ the TSS was almost exhausted. The result obtained in this study is in support of the report of Kumar et al. [32] who analyzed the physicochemical analysis of fresh and probioticated fruit juices with *Lactobacillus casei.*

### 3.1.1 Colour assessment of the soursop juice samples

The colour assessment of the juice samples is shown in Fig. 6. There were no significant differences ($P > 0.05$) in the colour of the Psop samples across the four weeks of storage. The colour of the Psop and Pcont samples ranged from 75.97 – 79.22 and 56.44 – 78.25. However there was significant ($P > 0.05$) difference in the colour of the Pcont samples with the lowest colour value obtained at 4th week of storage. Changes in colour of the Pcont juice samples during storage may be due to biodeterioration activity of some spoilage microorganisms in the product. Ability of the Psop samples to retain the colour may be due to the production of some bioactive metabolites by the probiotic starter which confer preservative properties on the samples.

### 3.2 Sensory Evaluation of the Probioticated and Unprobioticated Soursop Juice Sample at Different Weeks of Storage

The sensory evaluation of the Psop and Pcont soursop juice samples is shown in Table 1. The samples were acceptable and strongly liked in terms of sensory parameters in the 1st and 2nd week of storage. The Pcont samples were extremely disliked in the 4th week of storage.

The Psop samples did not show sensory differences for the 1st two weeks of storage. But after the second week, difference was perceived in color, aroma, taste and appearance. According to a consensus made with the panelists during sensory evaluation, it was determined that the main description that characterized the product were acidity and sweetness, with acidity being the attribute responsible for the sensory difference perceived by the panelists. This is in support of the work of Srivastava et al. [31] who developed a fermented whey and pineapple beverage using a mixed culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus.* LAB play an important role in food fermentation as the products obtained are characterized by hygienic safety, storage stability and attractive sensory properties. The preservative action of starter strains in food and beverage systems is attributed to the combined action of the range of antimicrobial metabolites produced during fermentation process [33-34].

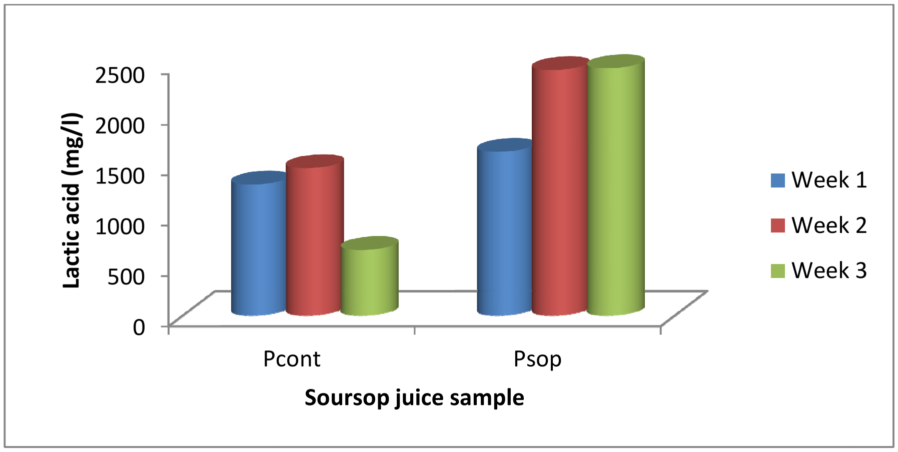


**Fig. 3. Lactic acid content of the soursop juice samples**

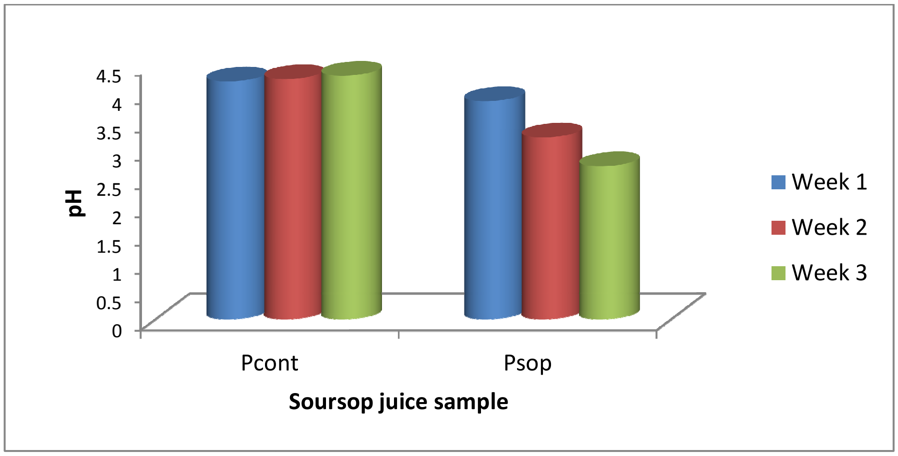


**Fig. 4. pH of the soursop juice samples**

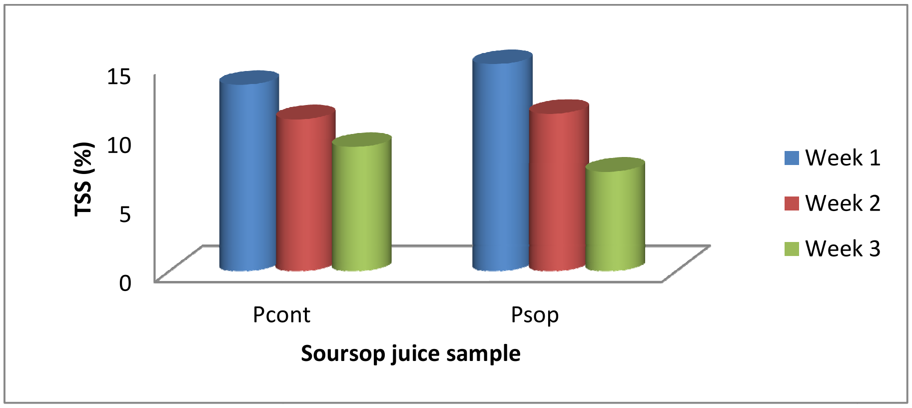


**Fig. 5. Total Soluble Solid (TSS) of the soursop juice samples**

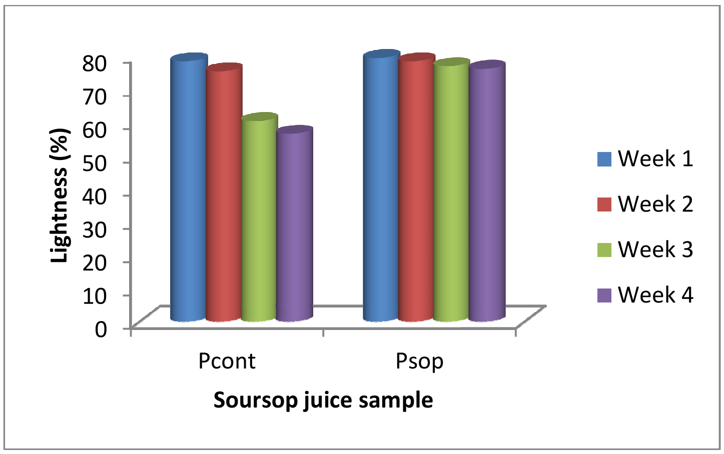


**Fig. 6. Colour assessment of the Pcont and Psop of the soursop juice samples**

**Table 1. Sensory evaluation of the soursop juice samples stored for 4 weeks**

| Tasters | Parameters | Storage time (weeks) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Psop | | | | Pcont | | | |
| | | Wk1 | Wk2 | Wk3 | Wk4 | Wk1 | Wk2 | Wk3 | Wk4 |
| 1. | Taste | 2.0[a] | 2.0[a] | 3.0[b] | 3.0[b] | 2.0[a] | 3.0[b] | 6.0[b] | 7.0[a] |
| 2. | Aroma | 2.0[a] | 2.0[a] | 3.0[b] | 3.0[b] | 2.0[a] | 4.0[a] | 6.0[b] | 7.0[a] |
| 3. | Colour | 2.0[a] | 2.0[a] | 3.0[b] | 3.0[b] | 2.0[a] | 4.0[a] | 6.0[b] | 7.0[a] |
| 4. | Appearance | 2.0[a] | 2.0[a] | 3.0[b] | 3.0[b] | 2.0[a] | 4.0[a] | 6.0[b] | 7.0[a] |
| 5. | Pcontrol | 2.0[a] | 2.0[a] | 7.0[a] | 7.0[a] | 2.0[a] | 4.0[a] | 7.0[a] | 7.0[a] |

*Scoring system: 1- Extremely liked 2= Strongly like 3= Liked 4= Moderately liked 5 = Dislike 6 = Strongly disliked 7=Extremely disliked*

The study of Hu et al. [35] showed that *Pediococcus pentosaceus* produced antioxidant effects on unsaturated fatty acids. The addition of competitive starter cultures to begin the fermentation process and for probiotication may be an effective method of inhibiting or controlling the growth of spoilage organisms, food-borne pathogens and preventing the formation of undesirable end-products.

### 3.3 Proximate Evaluation of the Psop and Pcont Soursop Juice Samples

Table 2 shows proximate evaluation of the Psop and Pcont soursop juice samples. The crude protein, crude fat, crude fiber content ranged from 0.58b – 0.65[a]%, 0.04[c] – 0.07[a] and 0.48[c] – 1.91[a]% for the Psop juice samples and 0.16[a]-0.60[b], 0.09[a]- 0.08[b], 1.96[a]-0.93[c] for Pcont juice

**Table 2. Proximate evaluation of Psop and Pcont juice samples stored at different time interval (weeks)**

| Proximate parameters (%) | Storage time (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | Psop | | | Pcont | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| Moisture | $91.89^c$ | $98.49^b$ | $98.85^a$ | $90.89^c$ | $91.49^b$ | $93.85^a$ |
| Crude protein | $0.58^b$ | $0.65^a$ | $0.65^a$ | $0.60^b$ | $0.61^a$ | $0.60^b$ |
| Crude fat | $0.07^a$ | $0.05^b$ | $0.04^c$ | $0.09^a$ | $0.08^b$ | $0.08^b$ |
| Crude fibre | $1.91^a$ | $0.77^b$ | $0.48^c$ | $1.96^a$ | $0.95^b$ | $0.93^c$ |
| Ash | $0.51^a$ | $0.41^b$ | $0.32^c$ | $0.87^a$ | $0.86^b$ | $0.86^b$ |
| CHO | $8.95^a$ | $7.40^b$ | $6.66^c$ | $10.95^a$ | $9.95^b$ | $9.06^c$ |

*Means with the same alphabets across the row are not significantly different from each other at 5% level of probability key 1-3= weeks CHO= Carbohydrate*

samples. The highest crude protein was recorded at week 2 and 3 of storage for Psop juice samples. There was a general reduction in the crude fat, crude fiber and carbohydrate content of the probioticated samples during storage.

The average moisture content of the fruit was $98.89^a$% and $98.85^a$% in the first and third week respectively but lower than the level of 82.8% reported by Waston and Preedy [36] and 81% reported by Onimawo, [37] for soursop fruits from Nigeria.

The soursop fruits juice had an average ash content of $0.51^a$ and $0.41^b$ in the first and third week of storage. This result is in contrary to the report of Morton [38] who worked on Soursop, the fruits of the warm climate.

The average fat content in soursop juice obtained in this study was $0.07^a$% this value is lower than the value of 0.22% for mango juice as reported by Othman and Mbogo [39]. The low level of fat in the fruits juice means that these fruits juice are not good source of energy and are recommended for loss or maintaining of weight, supply of nutrients and lowering of blood pressure [40].

## 4. CONCLUSION

In conclusion the probiotic soursop juice samples contain appreciable quantity of probiotic *Pediococcus pentosaceus* LBF2. The probiotic culture was viable during storage and contains appreciable level of lactic acid, low pH value and good colour. The probioticated juice samples (Psop) had good nutritional composition and were strongly accepted.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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