



Physicochemical, microbial and sensory properties of *Lactobacillus acidophilus* fermented soursop-beetroot (*Anona muricata* – *Beta vulgaris*) beverage

Jennifer Adanne Dike, Lucretia I Barber*, Monday O Akusu, Patience C Obinna Echem

Department of Food Science and Technology, Rivers State University, NKpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

Abstract

The effect of *Lactobacillus acidophilus* fermentation on the physicochemical, microbial viability and acceptability of soursop-beetroot beverages were evaluated. Soursop pulp and beetroot juice were blended in the ratios of: 100:0, 95:5, 90:10, 85:15, 80:20 and 0:100 (v/v) and labelled SBA, SBB, SBC, SBD, SBE, and SBF respectively. These were pasteurized and fermented with *Lactobacillus acidophilus* at 42°C for 24 h to produce potential probiotic beverages. Physicochemical, proximate and sensory properties of the beverages were evaluated. Viability of *L. acidophilus* during the fermentation period was also determined. pH decreased significantly ($p < 0.05$) and ranged from 5.75 – 6.43 and 3.64 - 3.96 for unfermented and fermented beverages. Within the fermented samples, the differences in pH were not significant ($p > 0.05$). Concentration of beetroot juice in the blends resulted in significant differences in TTA which ranged from 0.08 - 0.24% lactic acid. Viscosity ranged from 0.076 - 0.39 Pa S⁻¹. *L. acidophilus* counts in the blends after 24 h of fermentation ranged from log 6 to log 8 CFU/ml. The final counts for SBF and SBC were log 8.00 and 8.32 CFU/ml respectively. Degree of likeness for taste of the fermented blended samples: SBB, SBC and SBD was neither like nor dislike 5.20, 5.85 and 5.90 respectively. Overall acceptability ranged from 6.20 to 3.0 samples SBA and SBF, respectively. Final *L. acidophilus* counts in all the samples were greater than log 6.0. This indicates a potential for classification of the beverage as probiotic. However, storage studies to evaluate the viability of the organism and determination of other parameters that confer the probiotic status on a product are recommended.

Keywords: *Lactobacillus acidophilus*, probiotic, soursop, beetroot, fermentation, beverage

Introduction

Beverages have been defined simply as liquids intended for drinking and include alcoholic beverages, sugary carbonated drinks, teas, coffee, cocoa beverages, dairy beverages, health beverages, fruit and vegetable juices and drinks, soy beverages, and drinking water. They are mostly consumed to satisfy thirst, rehydrate the body or aid relaxation (Mullay, 2010) [13]. Most carbonated, fruit juices and drinks, fermented and non-fermented dairy beverages have high sugar content and their consumption have been implicated as risk factors in obesity, diabetes cardio vascular diseases and other health challenges. Demand for beverages with added health benefits and less sugar is on the increase as consumers become more health conscious. This consciousness has led to the development of the concept of functional foods. These constitute part of the normal diet and besides basic nutrients, provide additional health supporting physiological benefits and reducing the risk of chronic diseases such as cardiovascular disease, diabetes, obesity, gastrointestinal, joint health, enhanced immune functions and certain cancers (Sun-Waterhouse, 2011) [20]. Functional foods are also known as 'Food for Specified Health Use.' Functional foods include those that contain probiotics, prebiotics and phytochemicals such as flavonoids, anthocyanins etc. Probiotics are live microorganisms which when consumed in recommended quantities offers benefits such as improved gut health to humans. Some selected strains of *Bifidobacterium*, *Streptococcus*, *Lactobacillus*, *Lactococcus* and *Saccharomyces* have been shown to have probiotic qualities and are incorporated in food products due to their reputed

health benefits (Sharanya and Penchalarju 2016) [19]. prebiotics are indigestible polysaccharides which are selectively metabolized by probiotics in the colon thereby increasing the population of probiotics with attendant health benefits. Dairy products have been the traditional vehicles of delivering probiotics in the diet, however, there is increasing evidence that plant matrices such as fruit juices and vegetable milks (soy, almond etc.) also have the potential to promote the growth of probiotics. Thus, *Lactobacillus casei* 431 was used to produce acceptable synbiotic beetroot juice with cell counts of log 8cfu/ml (Gamage *et al.*, 2016) [9]. Fruit based juices are good sources of vitamins, minerals, and bioactive compounds such as polyphenols, flavonoids and beta carotenes which are essential for human health (Ana *et al.*, 2013) [2]. Functional juices and beverages are convenient means of providing nutrients and other beneficial substances to the body and have high appeal to consumers because they are mostly ready- to-drink (RTD) or are easy to reconstitute and serve. Blending two or more fruit or vegetable juices is expected to enhance their nutritive value, health benefits and in some cases, the organoleptic properties of the fruits (Deka and Sethi, 2001) [7]. Blending of the juices of different fruits, vegetable, or fruits and vegetables is expected to increase variety of these products as a result of new product development.

Soursop is a tropical fruit belonging to the family *Annonaceae*. It is valued for its pleasant, sub-acidic taste and unique aromatic flavor (Olagunju *et al.*, 2018) [14]. This fruit is an important source of vitamins, minerals, and dietary fiber. It is often eaten raw or occasionally extracted

with water and consumed as fresh soursop juice. The fruit is grossly underutilized due to its short shelf life as a result of rapid ripening which produces a soft and mushy fruit which is difficult to process and consume due to loss of eating quality. There is therefore, need to process it into value added products such as fermented beverages. Such products with extended shelf-life, improved nutrient content and added health benefits are in high demand by health conscious consumers. Processing will also minimize post-harvest losses and maximize its potential for use in food processing industries.

Beetroot (*Beta vulgaris*) is the taproot of the beet plant, also known as the table beet, garden beet, red or golden beet or informally simply as the beet. It is consumed mostly as cooked or raw vegetable. In the food processing industry, it is used as a natural colorant (betalins), in fruit juices, cookies etc. (Gamage *et al.*, 2016) [9]. It contains significant amounts of Vitamins C and B and has the potential to prevent and lower the risk of several chronic diseases such as cardiovascular, hypertension, obesity and diabetes due to its high antioxidant potential. (Dambalkar *et al.*, 2016) [6] Acceptable probiotic beverage had been produced from beets using *Pediococcus pentosaceus* (Akpeji *et al.*, 2017) [1] The health benefits and disease preventing potentials of fruit juices can be enhanced by blending two or more juices and through lactic fermentation by lactic acid bacteria such as *L. acidophilus* and *Pediococcus pentosaceus*. These organisms are considered probiotics and when present in foods in numbers greater than log 6, they confer several health benefits such as improved gut health, reduction and prevention of certain cancers and improvement of the immune system (Sharanya and Penchalarju 2016) [19] Interest in natural food preservation is on the increase and lactic fermentation is one of the methods with great potential especially in developing countries.

L. acidophilus although an animal species has been shown to grow very well in plant matrices

(Barber *et al.*, 2020) [5]. There are no reports of the use of *L. acidophilus* to produce a lactic fermented beetroot -soursop beverage. Thus, the aim of this study was to evaluate the physicochemical, microbial and sensory properties of *Lactobacillus acidophilus* fermented soursop-beetroot beverage.

Materials and methods

Materials

Soursop, beetroots and gum arabic

Soursop and beetroots were purchased from Mile Three Market, Port Harcourt, Rivers State, Nigeria. Gum Arabic was obtained from standard analytical grade chemical suppliers in Port Harcourt.

Fermenting microorganism and microbial medium

The fermenting microorganism used was probiotic species of *Lactobacillus acidophilus* (Nature source, UK). Microbiological media were: De Mann Rogosa Sharpe (MRS) agar and peptone water broth (Oxoid, UK).

Analytical reagents

Analytical grade reagents used included hydrochloric acid (HCL), calcium hydroxide, sodium hydroxide, etc. were obtained from the chemical store of the Department of Food Science and Technology, Rivers State University, Port Harcourt.

Methods

Extraction of soursop pulp

Soursop fruits were washed in portable water, sanitized with 70% ethanol and rinsed with distilled water. The fruits were peeled by hand and deseeded to obtain the pulp. The pulp was blended with water (1:4 w/v) by a modification of the method of Umme *et al.*, 2001) [21] an electric blender (Kenwood BLP610WH Model. United Kingdom). Soursop pulp produced was packaged in glass bottles, stored in a refrigerator and used within 30 min.

Table 1: Formulation of soursop-beetroot beverage

Sample	Sour-sop pulp: beetroot juice	Soursop pulp (ml)	Beetroot juice (ml)	Total volume (ml)	Sugar (g)	Gum Arabic (g)
SBA	100:0	300	-	300	20	20
SBB	95:5	285	15	300	24	20
SBC	90:10	270	30	300	25	20
SBD	85:15	255	45	300	28	20
SBE	80:20	240	60	300	32	20
SBF	0:100	-	300	300	40	20

SBA = 100% Soursop: 0% beetroot

SBB = 95% Soursop: 5% beetroot

SBC = 90% Soursop: 10% beetroot

SBD = 85% Soursop: 15% beetroot

SBE = 80% Soursop: 20% beetroot

SBF = 0% Soursop: 100% beetroot

Production of beetroot juice

Beetroot juice was produced using the method of (Emelike *et al.*, 2015) [8]. Briefly, mature beetroot was selected, washed thoroughly with portable water, rinsed, peeled, and sliced. The sliced beets were blended with Kenwood blender (BLP610WH Model. Havant, United Kingdom) with portable water in a 1:2 (w/v) ratio to aid juice extraction. The beetroot extract was filtered using a 4-layer muslin, the

juice obtained was stored in a glass bottle in the refrigerator and used within 20 min of production.

Formulation of soursop-beetroot beverage

Six samples of soursop-beetroot beverage were formulated by blending the soursop pulp and beetroot juice in ratios as shown in Table 1. Sugar was added to adjust the soluble solid content to 15°B and gum Arabic were added to aid fermentation and act as stabilizer, respectively.

Physicochemical analysis of *L. acidophilus* fermented soursop-beetroot beverage

Determination of pH

The pH of the samples was determined by AOAC method (AOAC 2012) [3]. Ten (10 ml) of each beverage sample was measured into a 100ml beaker. The electrode of the pH meter (Thomas Scientific, Germany) was submerged into each beverage sample and the pH read on the LCD screen after sufficient time was allowed for stabilization.

Determination total titratable acidity

Total titratable acidity was determined by the method of AOAC (2012) [3]. Ten milliliters (10 ml) of each soursop-beetroot sample was pipetted into Erlenmeyer flasks. Phenolphthalein (0.5 ml) was added and mixed thoroughly. Then the soursop-beetroot beverages were titrated against 0.1 M NaOH until the first tinge of pink appeared. TTA of the samples as percentage lactic acid was calculated as in equation 2:

$$\% \text{ TTA} = \frac{\text{Vol. NaOH} \times \text{NBase} \times \text{equivalent mean}}{\text{Vol. of Sample}} \times 100 \quad \text{Eq.1}$$

Determination of viscosity

The viscosity of the soursop-beetroot juice samples was determined using a rotary digital viscometer (NDJ-85, China) with spindle number 2 at 30rpm (ASTM D445, 2009).

Determination of syneresis index

The method of (Unla *et al.*, 2012) [22] was used to determine the syneresis index of the fermented beverages. Twenty (200) ml of each of the fermented soursop-beetroot beverage was centrifuged (L-600 China centrifuge) at 5000rpm for 10 min. The supernatant was weighed and syneresis index (SI) was calculated using equation 2:

$$\text{SI} (\%) = \frac{\text{Weight of Supernatant}}{\text{Weight of Sample}} \times 100 \quad \text{Eq.2}$$

Determination of total soluble solids (TSS)

A hand-held refractometer, (ATAGO) was used for this determination. The lens of the refractometer was cleaned using distilled water and dried with a lint-free cloth. A drop of the beverage was placed on the lens and closed. The percentage TSS was read from the scale of the refractometer by viewing the prism through the eye-piece. Total soluble solid was recorded as °Brix.

Total viable count of *L. acidophilus* in fermented soursop-beetroot beverages

The *Lactobacillus acidophilus* count was determined using the modified method reported by Hasani *et al.*, (2016). Ten (10) ml of each beverage instead of the 25ml used by Hassani *et al.*, (2016) [10] and MRS agar instead of MRS-bile salt agar was used for the enumeration of *L. acidophilus*. Ten ml from each sample was serially diluted in 9ml sterile peptone water and 0.1ml from the 10⁵ and 10⁶ dilutions were plated in duplicates on MRS agar using the spread plate method. The plates were incubated anaerobically at 42°C for 48h in an anaerobic jar with Oxoid anaerogen gas generating system. At the end of the incubation period, plates showing between 30 and 300 colonies were counted and the average number of organisms were expressed as colony forming units per ml (CFU/ml) using the formula:

Cell counts (CFU/ml) = (Average No. of colonies × Dilution Factor)/Volume plated. Eq. 3

Proximate composition of *L. acidophilus* fermented soursop-beetroot beverage

Moisture, fat, protein and ash, were determined using methods of AOAC (2012) [3]. Carbohydrate was calculated by difference between 100 and the sum of crude protein, fat, ash and moisture.

Determination of acceptability of *L. acidophilus* fermented sour-sop beetroot beverage

The degree of likeness of appearance, taste, aroma, mouthfeel and general acceptability of the soursop-beetroot beverages was carried out using a 9-point hedonic scale. A 20-member panel was selected from among students, lecturers and non-teaching staff of the Department of Food Science and Technology, Rivers State University who are familiar with non-dairy beverages. The samples (100ml) coded with 3-digits, were served to the panelists in transparent plastic cups in a well lit room. For panelists who did not wish to swallow the samples, an empty cup with a cover was provided for expectoration. A glass of portable water was provided to rinse the mouth between samples. Panelists were instructed to score the degree of likeness of the sensory parameters using the scale: 1 = Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5 = Neither like nor dislike, 6 = Like slightly, 7 = Like moderately, 8 = Like very much and 9 = Like extremely.

Statistical analysis

All data obtained were expressed as means of three independent trials. Means were subjected to analysis of variance (ANOVA) and separated using Turkey's multiple range test (SPSS version 24) at a significant level of (P=0.05).

Results and discussion

Physicochemical characteristics of *L. acidophilus* fermented soursop-beetroot beverage.

pH of fermented soursop-beetroot beverage

The pH of the samples before fermentation ranged from 5.75 – 6.43. (Table 2). There was significant difference (P=0.006) between the initial pH values of SBA, SBC, SBD, SBE and SBF. Plain beetroot juice has a lower acidity than soursop juice. The initial pH of beetroot juice reported in this study is however higher than that reported by (Ana *et al.*, 2013) [2] Fermentation however resulted in significant differences between the initial pH of all the samples and their final pH. The pH of the fermented samples ranged from 3.64 – 3.96 (Table 2). However, the concentration of beetroot juice did not result in any significant (P=0.279) difference between the final pH of all the samples. Sample SE (20% beetroot inclusion) had the lowest pH of 3.64. Parmjit and Shinde (2011) [16] reported pH of 3.91 in probiotic yoghurt fortified with aloe vera. Reduction in pH of fruits and vegetable beverages after fermentation is due to the metabolism of carbohydrates especially sugars by microorganisms present in the medium resulting in production of acids. The low pH of the samples will likely enhance the keeping quality of the product especially against pathogenic organisms.

Total titratable acidity of fermented soursop-beetroot beverage.

Final total titratable acidity (TTA) of the fermented blends ranged from 0.16 to 0.24% lactic acid (Table 2). Increased addition of beetroot juice up to 20% resulted in significant ($P=0.00013$) increase in the TTA of the samples. (Table 2). The highest value of 0.24% obtained in this study is less than the 0.53% reported for *L.acidophilus* fermented plain beetroot juice but higher than 0.23% reported for *L. casei* Yoon *et al.*, (2005) [2]. The principal acid produced by *L.acidophilus* is lactic acid and small amounts of formic acid (Parmjit and Shinde 2011) [16]. Total titratable acidity is the sum total of dissociated and non- dissociated acids in the system. The unblended samples SA and SF had the lowest TTA values of 0.16% as shown in Table 2. The acidity in fermented foods serve as preservative and also confers unique flavour to lactic fermented foods.

Viscosity of fermented soursop-beetroot beverage.

The viscosity of the soursop-beetroot juice beverages ranged from 0.39 to 0.76 Pa s⁻¹ (Table 1) Unblended fermented soursop beverage (SA) had the highest viscosity and the

viscosity of the blends decreased significantly ($P=0.008$) with increase beetroot juice. Shalaby and Mohamed (2022) [18] reported viscosity values of 970, 980 and 990 cp (0.97, 0.98 and 0.99 Pa. s⁻¹) for probiotic turnip juice fortified with 5,10 and 15% banana juice respectively. The decrease in viscosity with increase in beetroot juice concentration in the beverages is probably due to diluting effecting by beetroot juice since it is less viscous than the soursop puree. The viscosity of beverages is one of the principal determinants of their drinking quality.

Total soluble solids in fermented soursop-beetroot beverage

Sample SE had the highest total soluble solid content (12 °Brix). The TSS values for other samples ranged from 9.00 – 11.00 (°Brix) as shown in Table 1. The differences in the TSS of the samples were not significant ($P=0.1675$). Probiotic soursop juice produced with *Pediococcus pentosaceus* LBF2 was reported to have a TSS value of 15.00 (°Brix) (Akpeji *et al.*, 2017) [1] which is in the same range as those reported in this study.

Table 2: Physicochemical properties of fermented soursop-beetroot beverages

Sample	Soursop beetroot (v/v)	pH	TTA (%)	Viscosity (Pa.s ⁻¹)	Syneresis	TSS (°B)
SBA	100:0	3.87 ^a ±0.00	0.06 ^d ±0.01	0.076 ^a ±0.00	64.41 ^c ±3.54	9.00 ^a ±0.00
SBB	95:5	3.90 ^a ±0.14	0.08 ^{cd} ±0.01	0.068 ^b ±0.00	61.95 ^c ±6.65	10.00 ^a ±0.00
SBC	90:10	3.90 ^a ±0.00	0.12 ^{bc} ±0.00	0.053 ^c ±0.00	66.85 ^{bc} ±0.60	11.00 ^a ±0.00
SBD	85:15	3.96 ^a ±0.00	0.10 ^{ab} ±0.01	0.045 ^d ±0.00	66.77 ^{bc} ±0.00	10.00 ^a ±0.00
SBE	80:20	3.64 ^a ±0.00	0.24 ^a ±0.01	0.041 ^e ±0.00	76.58 ^b ±0.06	12.00 ^a ±0.00
SBF	0:100	3.85 ^a ±0.00	0.11 ^d ±0.00	0.039 ^f ±0.00	92.75 ^a ±3.40	9.50 ^a ±0.00

Values are means of triplicate determinations.

Means with the same superscript alphabets in the same column are not significantly different ($P>0.05$)

SBA = 100% Soursop: 0% beetroot

SBB = 95% Soursop: 5% beetroot

SBC = 90% Soursop: 10% beetroot

SBD = 85% Soursop: 15% beetroot

SBE = 80% Soursop: 20% beetroot

SBF = 0% Soursop: 100% beetroot

Viable counts of *L. acidophilus* in fermented soursop-beetroot beverage

The counts of *L. acidophilus* in the unfermented samples (inoculum size) was log 6.0 CFU/mL. After 24 h. fermentation, *L. acidophilus* increased significantly ($p<0.05$) in all the samples by 1 log cycle to log 7.40, 7.84, 7.40 and 7.96 (Samples SBA, SBB, SBD and SBE) respectively. However, counts in Samples SF and SBC increased by 2log cycles to log 8.00 and 8.32cfu/ml respectively. The increase in counts indicate that the beverages are suitable matrices to sustain the growth of *L. acidophilus*. Yoon *et al.*, (2005) [23] reported that *L. casei*, *L. plantarum* and *L. delbrueckii* attained counts of log 8.00 CFU/ml after 24 h fermentation of beetroot juice. Fruits and their products contain adequate amounts of metabolizable carbohydrates and other nutrients required for growth and proliferation of bacteria. The counts of greater than log 6.0 after fermentation confers a potential probiotic status on the fermented beverage with health benefits.

Proximate composition of fermented soursop-beetroot beverage.

Moisture content

Moisture content of the fermented beverages ranged from 86.62% (SC) to 90.05% (SBF) (Table 3). Addition of beet juice resulted in significant ($P=0.0290$) changes in the moisture content of the blends. The high moisture content of the blends indicate that these products are high moisture foods. The moisture content of the samples in this study is higher than the range r for fermented soy and almond milks blended with butterfly pea flower extracts. Soy-carrot-beetroot beverages with moisture content range of 89.31 – 92.10% were reported by Banigo *et al.*, (2015) [4]. The high moisture contents reported in the present study, is desirable in beverages because beverages contribute significantly to the intake of water by many individuals.

Table 3: Proximate composition (%) of *L. acidophilus* fermented soursop-beet beverage

Sample	Moisture	Crude protein	Ash	Fat	Carbohydrate
SBA	89.21 ^{ab} ±0.86	1.94 ^a ±0.00	0.50 ^b ±0.04	0.14 ^b ±0.00	8.2 ^f ±0.63
SBB	87.85 ^{bc} ±0.00	1.79 ^b ±0.00	0.38 ^c ±0.03	0.20 ^{ab} ±0.01	9.78 ^c ±0.21

SBC	86.62 ^c ±0.61	1.86 ^a ±0.00	0.30 ^d ±0.01	0.27 ^a ±0.00	10.95 ^a ±0.59
SBD	86.88 ^c ±0.00	1.94 ^a ±0.00	0.58 ^a ±0.02	0.20 ^{ab} ±0.00	10.40 ^b ±0.40
SBE	88.44 ^{abc} ±0.00	1.81 ^a ±0.00	0.50 ^b ±0.01	0.16 ^b ±0.01	9.09 ^d ±0.01
SBF	90.05 ^a ±0.34	0.97 ^c ±0.00	0.22 ^c ±0.01	0.15 ^b ±0.04	8.61 ^e ±0.38

Values are means of triplicate determinations ± SD.

^cMeans with the same superscript in the same column are not significantly different (P> 0.05).

SBA = 100% Soursop; 0% beetroot

SBB = 95% Soursop; 5% beetroot

SBC = 90% Soursop; 10% beetroot

SBD = 85% Soursop; 15% beetroot

SBE = 80% Soursop; 20% beetroot

SBF = 0% Soursop; 100% beetroot

Protein content

There was significant (P=0.0078) difference in protein content as indicated in Table 3 which showed a percentage protein ranged of 0.97 to 1.94%. Protein content range of 2.2 to 2.88% was reported in lactic fermented turnip juice containing 5 to 15% banana juice (Shalaby *et al.*, 2022) [18]. The values reported were also lower than those of probiotic beetroot juice (Pangha *et al.*, 2017) [15]. Fruit based beverages are generally low in protein content however, they provide useful nutrients such as vitamins, minerals phytochemicals and serve as vehicles to deliver probiotics.

Ash content

Ash content of the freshly produced beverage ranged from 0.22 to 0.58%. The values were significantly different (P=0.0292). Ash content is an indication of the amount of

minerals in a sample. Ash content is an indication of the amount of minerals in a sample. The ash content of the test samples was lower than 6.2% reported by Hasanni *et al.*, (2016) [10] for yoghurt supplemented with rice bran.

Carbohydrate content

There was a significant (P=1.24E-09) variation in the mean value for all the samples on the initial day of the analysis of carbohydrate. Sample SC had the highest carbohydrate content, while SB and SD had carbohydrate values that were not significantly different from each other (P=1.24E-09). SA had the lowest carbohydrate value of 8.21%. These values are higher than those reported for probiotic carrot juice by Rafiq *et al.*, (2016) [17], Carbohydrates provide energy for growth of microorganisms and their metabolism by *L. acidophilus* produce metabolites which include lactic acid with concomitant reduction in pH.

Table 4: Sensory evaluation of freshly fermented soursop beetroot beverage

Sample	Taste	Tartness	Aroma	Mouthfeel	Overall Acceptability
SBA	6.05 ^a ±0.71	6.00 ^b ±0.53	6.10 ^a ±0.26	5.65 ^a ±0.80	6.20 ^a ±0.40
SBB	5.20 ^c ±0.49	4.85 ^e ±0.61	4.75 ^d ±0.22	5.10 ^c ±0.44	5.75 ^b ±0.39
SBC	5.85 ^b ±0.64	5.85 ^c ±0.24	5.20 ^c ±0.48	5.55 ^a ±0.72	5.65 ^{bc} ±0.42
SBD	5.90 ^b ±0.20	6.25 ^a ±0.55	5.50 ^b ±0.37	5.20 ^{bc} ±0.48	5.50 ^{cd} ±0.61
SBE	4.90 ^d ±0.71	5.45 ^d ±0.57	5.10 ^c ±0.54	5.25 ^b ±0.61	5.40 ^d ±0.55
SBF	3.00 ^e ±0.44	4.25 ^f ±0.30	2.80 ^e ±0.43	3.15 ^d ±0.29	3.10 ^e ±0.51

Values are means of scores from 20 assessors ± SD.

Means having the same superscript alphabets in the same column are not significantly different (P> 0.05)

Sensory evaluation of freshly fermented soursop beetroot beverage

Sensory scores for the taste of freshly fermented samples ranged from 3.90 to 6.05 (dislike moderately to like slightly) for samples SBA and SF respectively. The degree of likeness for the taste of the fermented blended samples: SBB, SBC and SBD was neither like nor dislike (5.20, 5.85 and 5.90) respectively. The degree of likeness for taste between the samples decreased significantly (p<0.05) with increase in the concentration of beetroot juice. The decreased rating for taste could be as a result of the increase in beetroot concentration with resultant increase in earthy taste of these samples. The tartness of the fermented blends showed significant difference and was rated between dislike slightly 4.85 (SBB) to like slightly 6.25 (SBD). In this study the higher degree of likeness for tartness is associated with increased pH hence lower acidity. Tartness is a sharp sensation related to acidity. Juices with moderate tartness are desirable because they are refreshing and also reduce the sugar: acid ratio of beverages. The ratings for aroma of the samples ranged from 2.80 for fermented unblended beetroot juice to 6.10 for fermented unblended soursop beverage

(SBA and SBE) respectively. The lower ratings for aroma of the blended the blended beverages could be as a result of reduced aromatics. The rating for mouthfeel and overall acceptability of the fermented beverage blends were neither liked nor dislike (Table 4). The ratings for mouth feel could be related to the low viscosity and total soluble solids of the samples.

Conclusion

Lactic fermentation of blends of soursop and beetroot juices by *L. acidophilus* produced an acceptable beverage with pH in the range of 3.64 to 3.9. These acidic pH values correlated with the degree of likeness for tartness which is a major index of acceptability of lactic fermented beverages. The overall rating for acceptability of the beverage suggests that the beverage was liked slightly. The final *L. acidophilus* counts in all the samples were greater than log 6. This indicates a potential for classification of the samples as probiotic.

Storage studies to evaluate the viability of the organism and determination of other parameters that determine the probiotic status of a product are recommended.

Acknowledgements

The authors appreciate the technical assistance of Dr Friday Owuno of the analytical laboratory of Food Science and Technology, Rivers State University, Port Harcourt.

References

1. Akpeji SC, Adebayo Tayo BC, Sanusi JF, Alao S. Production and Properties of Probiotic Soursop Juice Using *Pediococcus pentosaceus* LBF2 as Starter. International Journal of Biochemistry Research and Review,2017;17(2):1-10.
2. Ana L, Cristina D, Nicolae C, Mircea O, Marcel A. Change in color and physicochemical quality of carrot juice mixed with other fruits. Journal of Agroalimentary Processes and Technologies,2013;19(2):241-246.
3. AOAC. Official Methods of Analysis, Association of official analytical chemists, 19th ed.; AOAC: Washington, DC, USA, 2012.
4. Banigo EB, Kiin Kabari DB, Owuno F. Physicochemical and sensory evaluation of soy/carrot drinks flavoured with beetroot. African Journal of Food Science and Technology,2015;6(5):136-140.
5. Barber LI, Osuji CM, Onuegbu NC, Ogueke CC. Quality Characteristics of Probiotic Soy Yoghurts with Enzyme Hydrolyzed African Breadfruit and Rice Additives. American Journal of Food Science and Technology,2020;8(6):233-241.
6. Dambalkar VS, Rudrawar BD, Poojari VR. Study of Physico-Chemical Properties and Sensory Attributes of Beetroot-Orange RTS Drink. International Journal of Science and Research,2016;4(10):589-594.
7. Deka BC, Sethi V. Preparation of mixed fruit juice spiced RTS beverages. Industrial. Food. Packer,2001;42(3):58-61.
8. Emelike NJT, Hart AD, Ebere CO. Influence of drying techniques on the properties, physicochemical and mineral composition of beetroot juice. IOSR Journal of Environmental Science, Toxicology and Food Technology,2015;9:20-26.
9. Gamage SM, Mhirani MKS, Perera ODAN, Weerahewa HLD. Development of synbiotic beverage from beetroot juice using beneficial probiotic *Lactobacillus casei* 431. Ruhuna Journal of Science,2016;7:64-69.
10. Hasani S, Khodadadi I, Ali Heshmati A. Viability of *Lactobacillus acidophilus* in rice bran-enriched stirred yoghurt and the physicochemical and sensory characteristics of product during refrigerated storage. International Journal of Food Science and Technology,2016;51:2485–2492.
11. Hutabarat DJC Irene. Chemical and Physical Characteristics of Fermented Beverages from Plant-Based Milk with the Addition of Butterfly Pea Flower (*Clitoria ternatea* L.) Extracts
12. IOP Conf. Series: Earth and Environmental Science 794 IOP Publishing doi:10.1088/1755-1315/794/1/012140. 4th International Conference on Eco Engineering Development 2020 IOP Publishing 2021
13. Mullay M. Dictionary of Food Science and Technology (2nd edition), Reference Reviews,2010;24(6):31-32.
14. Olagunju AL, Omoba OS, Enjuigha VN, Aluko RE. Development of value-added nutritious crackers with high antidiabetic properties from blends of acha (*Digitaria exilis*) and blanched pigeon pea (*Cajanus cajan*). Food Science and Nutrition,2018;6:1791-1802.
15. Panghal A, Virkar K, Kumar V, Anju BD, Gat Y, Chhikara N. Development of Probiotic Beetroot Drink. Current Research in Nutrition and Food Science,2017;5(3):257-262.
16. Parmjit SP, Shinde C. Effect of Storage on Syneresis, pH, *Lactobacillus acidophilus* Count, *Bifidobacterium bifidum* Count of Aloe vera Fortified Probiotic Yoghurt. Current Research in Dairy Sciences,2011. ISSN 1994-5434 / DOI: 10.3923/crds.
17. Rafiq S, Sharma V, Nazir A, Rafia Rashid R, Sofi SA, Nazir F, et al. Development of Probiotic Carrot Juice. Journal of Nutrition and Food Sciences,2016;6:4-9.
18. Shalaby HS, Mohamed AS. Study of the Physicochemical, Sensorial, Microbiological, and Antioxidant Properties of Probiotic-Fortified Turnip Juice During Storage. Bulletin of the National Nutrition Institute of the Arab Republic of Egypt,2022;59:105-122.
19. Sharanya RD, Penchalarju M. A review of different types of functional foods and their health benefits. International Journal of Applied and Natural Sciences,2016;5(3):19–22. (IJANS)ISSN(P): 2319-4014; ISSN(E): 2319-4022, Apr – May
20. Sun Waterhouse D. The Development of Fruit-Based Functional Foods Targeting the Health and Wellness Market: A Review. International Journal of Food Science and Technology,2011;46:899-920.
21. Umme A, Bambang SS, Salmah Y, Jamilah B. Effect of pasteurisation on sensory quality of natural soursop puree under different storage conditions. Food Chemistry,2001;75:293-301.
22. Unla MU, Ucan F, Şener A, Dincer S. Research on antifungal and inhibitory effects of DL-Limonene on some yeasts. Turkish Journal of Agriculture and Forestry,2012;37:576-582.
23. Yoon *et al.* Beneficial beetroot juice. International Journal of Food Science and Technology,2016;51:2485–2492.
24. Yoon KY, Woodams EE, Hang YD. Fermentation of beet juice by beneficial lactic acid bacteria. LWT -Food Science and Technology,2005;38:73-75.