Antioxidant Activity and Antimicrobial Properties of Yoghurt Produced from Cow Milk and Soursop Juice Blends

Okoronkwo, N.C., Mbaeyi-Nwaoha, I.E., Eze, C.M., Azuka, C.E. and Agbo, E.C.

Department of Food Science and Technology, University of Nigeria, Nsukka, Enugu State, Nigeria

***Corresponding author:** ngozi.okoronkwo@unn.edu.ng **Phone number:** 08064645849

ABSTRACT

Background: *The increasing demand for probiotic-rich and functional food products has led to the exploration of alternative sources for yoghurt production.*

Objective: *This study evaluated the antioxidant and antimicrobial properties of yoghurt produced from full cream milk and soursop juice.*

Methodology: *The yoghurt samples were produced in the following ratios: 100% cow milk and 0% soursop juice (YC_0), which served as the control; 50% cow milk and 50% soursop juice (YS); and 0% cow milk and 100% soursop juice (SC_0). The chemical, antioxidant, and antimicrobial properties of the yoghurts were determined.*

Results: *The pH, total titratable acidity, and total soluble solids ranged from 3.80 to 4.55, 1.06-1.44%, and 20.70-31.60%, respectively. Syneresis value ranged from 0.00 to 26.70%, with sample SC_0 having the highest value. The addition of soursop juice to the yoghurt significantly ($p<0.05$) increased the syneresis value. There was a significant ($p<0.05$) difference among the samples in their viscosity concentration, ranging from 1191.50- 39.75 mPa · s. The DPPH IC-50 and total phenolic content increased significantly as soursop juice was added, and values ranged from 5.51-25.36mg/ml and 28.24-73.6 mgGAE/100g, respectively, while Vitamin C content ranged from 2.11 -18. 11 mg/100g, and increased significantly as soursop juice was added. Sample SC_0 had the highest antibacterial activity (20.67 mm) at 100% concentration, while sample YC0 had the lowest antibacterial activity (10 mm) at 100%. The minimum inhibitory concentration varied from 12.5-25 %.*

Conclusion: *This study showed that the use of soursop juice in yoghurt production improved the antioxidant and antimicrobial properties of yoghurt.*

Keywords: Yoghurt, Antioxidant properties, Antimicrobial status, Soursop juice, Functional beverages

Doi: https://dx.doi.org/10.4314/njns.v47i2.6

INTRODUCTION

Plant milks are suspensions of dissolved and disintegrated plant material in water that has the look of cow's milk (1). Plant-based milk alternatives are emerging functional beverages (2). This is because beverages are no longer just thirst quenchers in today's world; consumers look for specific functionality that fits into their lifestyle. Growing knowledge of the relationship between nutrition and health has resulted in a high level of acceptance and desire for foods and food products that improve health by supplying essential nutrients. This knowledge has increased demand for functional foods, especially among children and other high-risk groups. Functionality in these beverages may address various needs such as boosting energy, combating aging, fatigue and stress, lower cholesterol, immune system support, reducing bloating, anti-inflammatory properties, probiotics for gut health and potential anti-cancer effects. Yoghurt is a cow milk fermented product, having several health benefits (3). It is high in protein, vitamins, minerals, and omega-3 fatty acids (4). In the production of yoghurt, milk is fermented by a defined bacterial culture under a controlled atmosphere, which modifies milk contents technologically and induces characteristic smell, taste, texture, colour, and flavour (5). The texture and distinct tangy flavour of yoghurt are caused by the fermentation of milk protein by lactic acid bacteria (6). The lactic acid producing bacteria strains that have been used for the culturing of yoghurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in combination with various optional dairy ingredients such as cream, milk and skim milk (7). Over the past decade, studies have shown that probiotic bacteria like *Bifidobacterium* and *Lactobacillus* are beneficial to health. *Lactobacillus plantarum* is a facultative heterofermentative LAB with a wide range of uses. Its dual role as an indigenous human gut inhabitant and safe starter culture in food fermentation makes it a preferred candidate for the development of novel functional products (8).

Global food consumption has significantly driven an increase in food production due to the growth of the global population. There has been a rise in protein consumption in high-income countries over the last 50 years (9). Meat, eggs, milk and dairy products account for most of this increase, rising from 39 to 52g per capita between 1961 and 2011 (10). Increased consumer awareness regarding environmental and health impacts of food production has further led to a decline in demand for animal-derived food products in industrialized countries (11). Many food producers and researchers are now focusing on developing healthy and sustainable alternative plant-based food products as a result of the increased awareness to meet current consumer expectations and environmental concerns (12). Plant-based fermented beverages have received much attention recently, because of their health benefits and sustainable food production (13). Applying fermentation to plant-based matrices has been found to be a safe and efficient biotechnological way to improve their functional, sensory, technical, and nutritional qualities (14), thereby meeting consumer and food industry demand. Due to lactose intolerance or allergies to dairy milk proteins and increasing awareness regarding the potential benefits of plant-based products, consumers are switching to plant-based yoghurts (13).

Plant-based yoghurt is typically made through aqueous extracts of legumes, cereals, nut flours, pseudo-cereals, and oilseeds. A variety of basic ingredients such as soy, oats, legumes, fruits, and coconut can be used to make plant-based yoghurt.

Soursop (*Annona muricata*) is a juicy, acidic, and aromatic tropical fruit with many therapeutic and nutritive properties (15). It belongs to the *Annonaceae* family. It contains nutrients such as vitamins, minerals, fibres, and carbohydrates. It is quite high in vitamins C, B1, and B2 as well as fructose (16). Soursop is considered a healthy fruit because of its bioactive compounds such as acetogenins, alkaloids, flavonoids, terpenoids, carotenoids, polyphenols, and tannins, among others. Soursop is still very much underutilized, although it contains valuable bioactive compounds which have many therapeutic and nutritive properties. Soursop juice-based yoghurt would create a new market segment by adding diversity to the already existing product. Therefore, the production of yoghurt from soursop juice was carried out to find alternative uses of soursop in the probiotic food product market and to determine the antioxidant activity and antimicrobial status of the formulated yoghurt.

MATERIALS AND METHOD

Sample Procurement and Preparation

The soursop (*Annona muricata Linnaeus*) was purchased from Opi Market, Nsukka, Enugu State. The full cream powdered milk, analytical lactose, and sucrose were purchased from Ogige Market, Nsukka, Enugu State. The starter culture used was Direct Vat Set, which was purchased from Onitsha Main Market, Anambra State, and *Lactobacillus plantarum* (B1848), which was gotten from the United States Department of Agriculture (USDA).

Production of Soursop Juice

The method described by (11), with slight modification, was used. The soursop fruits were sorted to remove the bad ones, after which they were washed and peeled. Soursop juice was extracted using a juice extractor and pasteurized at 85^0C for 3 minutes. It was then cooled.

Production of Yoghurt from Full Cream Milk and Soursop Juice

The yoghurt was produced using the method described by (5) with slight modification. In formulating the yoghurt samples, full cream milk and soursop juice were measured and blended in proportions of 100:0(YC_0), 50:50(YS), and 0:100(SC_0), full cream milk to soursop juice, respectively. The 100% full-cream milk yoghurt served as the control. Analytical lactose was added to samples YS and SC_0, while sucrose was added to all the samples, and the mixtures were homogenized properly. The lactose served as a source of fermentable sugar for the microorganisms, while the sucrose served as a sweetener. The mixture was then pasteurized at 80°C for 15 minutes. The samples were cooled to a temperature of 45°C and inoculated with 5 mL of the starter culture DVS for YC_0 and *Lactobacillus plantarum* for YS and SC_0, and mixed thoroughly. The plastic containers were covered and incubated in a warm environment that maintained the temperature at 45°C for 12 hours. After incubating at 45 °C for 12 hours, a set yoghurt was obtained.

Plate 1: Pictorial Representation of Soursop Fruit and Soursop Juice

Determination of the Physicochemical Properties

Determination of pH

A standard pH meter (model 20 pH conductivity meter, Denver Instrument, United Nations Inventory Database) was standardized using buffer solutions of pH 4.4 and 9.0. The pH electrode was dipped into the yoghurt, and after a few minutes of equilibrium, the pH of the yoghurt sample was taken (18).

Total Titratable Acidity

The titratable acidity was measured by titrating 15ml of the yoghurt with 0.1 M sodium hydroxide until the substance reached a pH value of 8.2 corresponding to the end-point of phenolphthalein. The total titratable acidity of the samples was calculated as given in equation (1).

Titratable acidity =

$$\frac{Titre\ value \times M \times 90}{Volume\ of\ sample} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where M= molar concentration of NaOH

Determination of Total Solids

Total solids content of the yoghurt samples was determined gravimetrically using the method described by (18).

Determination of Viscosity

The method of (19) was adopted. One hundred and fifty milliliters (150ml) of the yoghurt was used and stirred mechanically for 3 hours at room temperature. Brookfield Digital Viscometer Model DV-II was used to measure the viscosity.

Determination of Syneresis

The syneresis (%) of yoghurt samples was determined by a centrifugation procedure using a modified version as described by (20).

Determination of Antioxidant Properties

Determination of DPPH Free Radical Scavenging Activity

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity was measured using a modified method by (21). Briefly, 150 µL of the sample was mixed with 750 µL of 0.1 mM DPPH solution (Sigma-Aldrich (St. Louis, MO, USA)) in ethanol. After 30 min, the solution was centrifuged at 14,240 × g for 1 min. The absorbance of the supernatant was measured at 517 nm. DPPH radical scavenging activity was calculated using equation (2) as follows:

% Inhibition of DPPH absorbance A =

$$\frac{Ac - As}{Ac} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Ac = Absorbance of the control
As = Absorbance of the aliquots

Determination of Total Phenolic Content

The total phenolic content (TPC) was measured using the modified method of (22). Briefly, 50 µL of food sample was mixed with 1 mL of 2% Na_2CO_3. After 5 min, 50 µL of 50% Folin-Ciocalteu reagent was added to the mixture. After 30 min, the absorbance was measured at 750 nm. TPC was expressed as mg gallic acid equivalent (GAE)/ml.

Determination of Vitamin C

This was done according to the method of (19). About 5 g of the sample and 2.5 ml of 20% meta-phosphoric acid were used as a stabilizing agent and diluted with distilled water. Ten milliliters of the solution and 2.5 ml of acetone were added. The absorbance reading at 264 nm, using a UV spectrophotometer, gives the vitamin C content.

Determination of Antimicrobial Property

The antimicrobial status of the formulated yoghurt against *Escherichia coli* (*E. coli*) was determined, following the (23) guidelines. The inhibitory zone diameter (IZD) and the minimum inhibitory concentration (MIC) were measured.

Statistical Design

The statistical design was laid down in a completely randomized design (CRD). Duncan's new multiple range test was used for mean separation using statistical product for service solution (SPSS) version 25.0, and the significance was accepted at $p<0.05$. Plate 1 to 4 shows the pictorial samples of the formulated yoghurt.

Plate 2: Sample YS (50% Cow Milk and 50% Soursop Juice)

Plate 3: Sample SCO (0% Cow Milk and 100% Soursop Juice)

Plate 4: Sample YC0 (100% Cow Milk and 0% Soursop Juice)

RESULTS

Physicochemical Properties of Yoghurt Samples Produced from Soursop Juice

Table 1 shows the physicochemical properties of the formulated yoghurt. The pH of the formulated yoghurt samples was within the range of 3.80 to 4.55. Samples YC_0 and YS differed significantly ($p<0.05$) from SC_0. Sample YS had the highest pH value (4.55±0.07), followed by YC_0, while SC_0 had the lowest value (3.80±0.00). Conversely, total titratable content of the yoghurt samples ranged from 1.06 to 1.44%. There was a significant difference ($p<0.05$) between the samples. Total titratable acidity was significantly highest in Sample SC_0 (1.44±0.05) and lowest in YS (1.06±0.03). Regarding total soluble solids, the samples ranged from 20.70% to 31.60%. Sample YS recorded the highest value (31.60±0.28), while sample SC_0 had the lowest (20.70±0.14). There are significant differences ($p<0.05$) between the samples. The viscosity of the yoghurt samples showed significant ($p< 0.05$) variations and ranged from 39.75 to 1191.50 mPa.s. Viscosity was highest in YC_0 (1191.50±1.70), followed by YS (384.20±0.42), while SC_0 had the lowest value (39.75±0.49). The values of syneresis of the yoghurt samples ranged from 0.00 to 26.70%, and they varied significantly ($p< 0.05$). The syneresis value was highest in SC_0 (26.70±0.14), the highest value, while YC_0 recorded no value for Syneresis (0.00±0.00). Overall, sample YC_0 stood out with the highest values for viscosity, while sample YS stood out with the highest values for pH, total soluble solids, and SC_0 stood out with the highest values for total titratable acidity and Syneresis.

Table 1: The Physicochemical Content of Yoghurt Samples Produced from Cow Milk and Soursop Juice

Sample	pH	Total titratable acidity (%)	Total soluble solids (%)	Viscosity (mpa.s)	Syneresis (%)
YC_0	$4.50^a±0.00$	$1.32^b±0.05$	$26.20^b±0.28$	$1191.50^a±1.70$	$0.00^c±0.00$
YS	$4.55^a±0.07$	$1.06^c±0.03$	$31.60^a±0.28$	$384.20^b±0.42$	$16.30^b±0.28$
SC_0	$3.80^b±0.00$	$1.44^a±0.05$	$20.70^c±0.14$	$39.75^c±0.49$	$26.70^a±0.14$

Values are means ± standard deviation of 2 replicate determinations.
Key: YC0-yoghurt with 100% cow milk and 0% soursop juice; YS- yoghurt with 50% cow milk and 50% soursop juice; SC0- yoghurt with 0% cow milk and 100% soursop juice

Antioxidant Properties of Yoghurt Samples Produced from Soursop Juice

Table 2 shows the antioxidant properties of yoghurt samples produced from cow milk and soursop juice. From the table, sample YC_0 had the lowest DPPH IC-50 value (5.51), while sample SC_0 had the highest DPPH IC-50 value (25.36), followed by sample YS (16.13). Total phenolic content ranged from 28.24 to 73.60 mgGAE/100g. The analysis revealed significant differences ($p<0.05$) among the samples. Sample YC_0 recorded the lowest value (28.24±1.01), while sample SC_0 had the highest value (73.60±1.39), followed by YS (49.61±0.23). The vitamin C content of the yoghurt samples ranged from 2.11 to 18.11 mg/100g. Vitamin C content was significantly lowest in YC0(2.11±2.73), while YS (11.01±0.73) and SC_0 (18.11±0.00) had comparably high values. Overall, sample SC_0 contained the highest values of total phenolic content, DDPH, and vitamin C content, while YC_0 contained the lowest values across the analysis.

Table 2: Antioxidant Properties of Yoghurt Samples Produced from Cow Milk and Soursop Juice

Sample	DPPH IC-50 values (mg/ml)	Total Phenolic Content (mgGAE/100g)	Vitamin C (mg/100g)
YC_0	5.51	$28.24^c±1.04$	$2.11^c±2.73$
YS	16.13	$49.61^b±0.23$	$11.01^b±0.73$
SC_0	25.36	$73.60^a±1.39$	$18.11^a±0.00$

Values are means ± standard deviation of 2 replicate determinations.
Key: YC0-yoghurt with 100% cow milk and 0% soursop juice; YS- yoghurt with 50% cow milk and 50% soursop juice; SC0- yoghurt with 0% cow milk and 100% soursop juice

Antimicrobial Properties of Yoghurt Samples Produced from Soursop Juice

Table 3 and Figure 1 show the antibacterial activity indicated by inhibition zone diameter (mm) and the minimum inhibitory concentration (%).

The inhibition zone diameters recorded with agar diffusion are presented in Table 3. From the table, the diameter of the inhibition zone was wider for sample SC_0 than for YS and YC_0. The result showed that all the samples were effective against *E. coli* at

100% concentration, but at other concentrations (80%, 60%, 40%, and 20%), only sample SC_0 exhibited antimicrobial activity. At 100% concentration, sample SC_0 showed the highest antibacterial efficacy, while sample YC_0 showed the lowest antimicrobial activity, characterized by the presence of zones of growth inhibition with diameters of 20.67 mm and 10 mm, respectively. At 80%, 60%, 40%, and 20% concentrations, only SC_0 showed an inhibitory effect characterized by the presence of zones of growth inhibition with diameters of 14 mm, 13.33 mm, 11.33 mm, and 10.67 mm, respectively.

Figure 1 shows the minimum inhibitory concentration (MIC) values of the yoghurt samples in percentage. The MIC values of the formulated yoghurt samples, YC_0, YS, and SC_0, are 25%, 20%, and 12.5%, respectively. The lowest MIC value, which led to very high antimicrobial susceptibility against *E. coli,* was 12.5% for sample SC_0. This shows that sample SC_0 had the highest inhibitory effect against *E.coli* since its MIC value was the lowest. Minimum inhibitory concentration is the lowest concentration of an antibiotic at which bacterial growth is completely inhibited.

Table 3: Antibacterial Activity of the Yoghurt Samples Produced from Cow Milk and Soursop Juice Indicated by Inhibition Zone Diameter (Mm)

Samples	Inhibition zone diameter (IZD), measured in mm, at the concentration gradients (%)					Control (Gentamicin)
	100%	80%	60%	40%	20%	10μg/ml
YC_0	10 mm	-	-	-	-	30.67 mm
YS	15 mm	-	-	-	-	36.67 mm
SC_0	20. 67 mm	14 mm	13.33 mm	11.33 mm	10.67 mm	37.33 mm

Values are means ± standard deviation of 2 replicate determinations.
Key: YC0-yoghurt with 100% cow milk and 0% soursop juice; YS- yoghurt with 50% cow milk and 50% soursop juice; SC0- yoghurt with 0% cow milk and 100% soursop juice

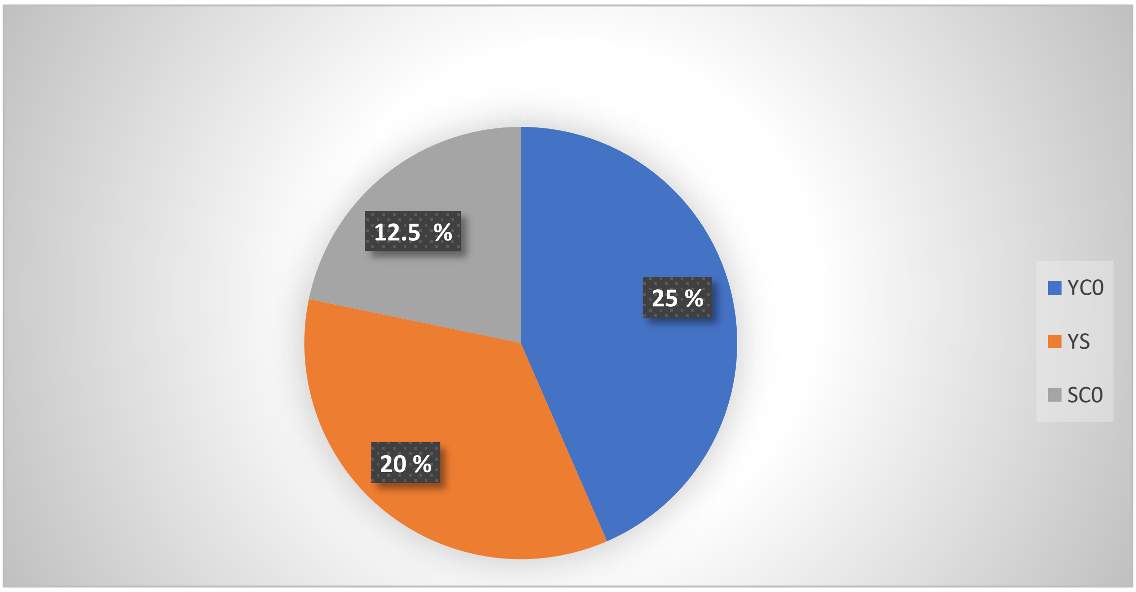

Figure 1: Minimum Inhibitory Concentration (MIC %) of the Formulated Yoghurt Samples Against *E. coli*.
Key: $YC_{0=}$ yoghurt with 100% cow milk and 0% soursop juice; YS= yoghurt with 50% cow milk and 50% soursop juice; SC0=yoghurt with 0% cow milk and 100% soursop juice

DISCUSSION

The pH values obtained in this study were in accordance with FDA specifications (4.6 or lower) for the pH of the yoghurt samples (25). The pH values reported by (6) were within the range of 4.31 to 4.60, which are similar to the pH values of YC_0 and YS in this research. The lower pH of SC_0 is due to the low pH of soursop juice. (18) reported 3.85 for the pH of soursop juice. Table 1 showed that the Titratable acidity of the samples decreased with an increase in pH values. Titratable acidity of 0.6% is the minimum value required by the Food

Standards Code for plain yoghurt (26). The FDA requires yoghurt to have a Titratable acidity of not less than 0.70%. However, all the yoghurt samples meet the total acidity standards of the USA, which is 0.9%, and that of Australia and New Zealand, which is not less than 0.7% (27). The TTA values obtained in the present study are higher compared to the TTA values obtained by (6) and (28), which are between 0.69-0.83% in flavoured yoghurt produced from graded levels of soursop pulp, and 0.85-0.88% in flavoured yoghurt produced from graded levels of sweet variety of African bush mango 'ugiri' juice and pulp, respectively. Also, the TTA of soursop-incorporated set yoghurt (0.70%) developed by (29) and that of soursop-incorporated stirred yoghurt (0.62%) developed by (30) are lower in value than the result obtained in the present study. The high TTA value of SC_0 is due to the inherent acidity of soursop. (31) reported that just 3-4 days after harvesting of ripe soursop, malic acid increased by sevenfold, ascorbic acid increased by 11-fold, and citric acid increased by threefold.

The total solids increased with an increase in the concentration of the soursop juice in yoghurt. The addition of soursop juice to yoghurt increased the total solids content of sample YS. The total solids values (20.74 to 23.22%) of flavoured yoghurt produced from graded levels of soursop developed by (6) and 13.85 to 14.24% of flavoured yoghurt from graded levels of sweet variety of African bush mango "ugiri" juice and pulp are lower than the results in the present study. (32) also reported a total solids value of 16.5% in yoghurt partially substituted with soursop juice. The low total solids content of sample SC_0 may be due to the high moisture content in soursop juice, and soursop juice has a lower total solids content compared to the cow milk used.

The viscosity of the cow milk yoghurt (YC_0) was higher than that of the other samples (1191.50 mPa.s.) because of the increased interactions between the molecules of milk due to casein micelles and whey proteins in milk. The viscosity dropped with the addition of soursop juice. This could be due to plant-based yoghurt's hydrophobic and electrostatic interactions, which are thought to be weak physical bonds (33).

Syneresis is a major visible issue in commercial yoghurt manufacturing, which leads to accumulation of whey on the surface of the gel, and it is considered a textural defect in yoghurt. This can lead to poor consumer acceptance of the product. The higher Syneresis value observed in yoghurts containing soursop juice is in agreement with the findings of other studies (15) in yoghurt incorporated with various *Annona species* pulp. The low pH of Sample SC_0 may have led to the contraction of the casein network and a higher level of syneresis.

The DPPH assay is considered a simple method that gives information on the radical scavenging activity of the antioxidant substance existing in a sample. IC-50 values are used as an indicator to evaluate the DPPH radical scavenging activity of the yoghurt samples. It is the concentration of the sample required to scavenge 50% of the DPPH free radical. The ability of antioxidant activity is generally divided into 4 categories: powerful antioxidants with IC-50 values between 0-50 mg/ml, potent antioxidants with IC-50 values of 50-100 mg/ml, moderate antioxidants with IC-50 values of 100-150 mg/ml, and weak antioxidants with IC-50 values between 151-200 mg/ml (34). The smaller the IC-50 value, the higher the scavenging rate (35). From the results obtained in Table 2, all the samples are powerful antioxidants, with sample SC_0 exhibiting the highest antioxidant activity, followed by YS. The high DPPH radical scavenging activity could be a result of the presence of phenolic content and vitamin C in soursop fruits. Fermentation leads to the release of phenolic acids and improved bioaccessibility and colonic metabolism of phenolic acids in fermentation (36).

There was an increase in total phenolic content as more soursop juice was included. This could be as a result of the phenolic content present in soursop fruit. Also, the increment may be due to the activities of enzymes that lead to the liberation of bound polyphenolic compounds during fermentation (37). (38) also reported an increase in total phenolic content in set yoghurt fortified with passion fruit juice. Also, the addition of strawberry pulp was shown to enhance the total phenolic content and antioxidant properties of yoghurt (39).

There was an increase in the vitamin C content with an increase in soursop juice. This could be due to the high vitamin C content in soursop. Vitamin C is one of the best-known natural antioxidants, essential for the proper functioning of the human body. The result of the vitamin C content is similar to the result obtained by (6). There was a significant ($p< 0.05$) difference among the samples. According to (16), soursop has a vitamin C content of 29.6mg/100g. However, it was reduced when the samples were subjected to a pasteurization temperature of above 80°C and this could be attributed to the degradation of vitamin C at a higher temperature.

The result of the antibacterial activity, as indicated by the inhibition zone, indicated that *E. coli* was more sensitive to sample SC_0. The diameter of the inhibition zone formed depends on the concentration of metabolite compounds. The higher the concentration of the metabolite compound formed by the sample, the stronger the ability to suppress the growth of pathogenic bacteria (40).

An inhibition zone diameter > 20mm is categorized as powerful; 10- 20 mm diameter is categorized as potent; 5- 10 mm is categorized as intermediate, and < 5mm is categorized as weak (34). This shows that SC_0 exhibited a powerful antibacterial efficacy, while YS and YC_0 are categorized as potent and intermediate antibacterial efficacy, respectively. This could be attributed to the antimicrobial effect of the soursop. Scientific studies have shown that soursop contains a class of compounds known as bioactive acetogenins of *Annonaceae*, which have antimicrobial properties (41,42). Also, soursop contains bioactive chemicals called alkaloids (annonaine, asimilobine, liriodenirine, nornuci-ferine, etc.) that target the outer and plasma membranes of bacteria to produce broad-spectrum antibacterial activity (43).

The exhibition of high antibacterial activities of soursop is attributed to the presence of terpenoids, flavonoids, and alkaloids (43). Also, the presence of bacteriocins, hydrogen peroxide, or lactic acid might be responsible for the inhibitory activity. This is in line with this study; the data from this study showed that the yoghurt samples containing soursop juice exhibit enhanced antibacterial activity against *E. coli*.

CONCLUSION

Stirred yoghurt samples were produced from soursop juice in varying ratios. The pH and total acidity showed an inverse relationship. The pH and viscosity decreased as soursop juice was added, while syneresis increased with the inclusion of soursop juice. The vitamin C content, total phenol, and DPPH of the yoghurt increased with the inclusion of soursop juice. The addition of soursop juice enhanced the antioxidant and antimicrobial properties of the yoghurt. Therefore, the inclusion of soursop juice in yoghurt increased the functionality of yoghurt.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of the United States Department of Agriculture (USDA), the Agricultural Research Service, and the ARS Culture Collection Center, USA, who provided the microbial culture used in this research work.

REFERENCES

1. Mäkinen, O. E., Wanhalinna, V., Zannini, E. & Arendt, E. K. (2015). Foods for special dietary needs: non-dairy plant-based milk substitutes and fermented dairy-type products. *Critical Reviews in Food Science and Nutrition*, 56(3): 339-349.
2. Velangi, M. & Savla, M. (2022). Role of plant-based milk alternatives as a functional beverage: a review. *International Journal of Health Sciences and Research*, 12(11): 273-281.
3. Kaur, R., Kaur, G., Rima, Mishra, S. K., Panwar, H., Mishra, K. K. & Brar, G. S. (2017). Yogurt: a nature's wonder for mankind. *International Journal of Food Fermentation*, 6(1): 57-69.
4. Weerathilake, W. A. D. V., Rasika, D. M. D., Ruwanmali, J. K. U. & Munasinghe, M. A. D. D. (2014). The evolution, processing, varieties and health benefits of yoghurt. *International Journal of Scientific and Research Publications*, 4(4):1-10.
5. Aswal, P., Shukla, A. & Priyadarshi, S. (2012). Yoghurt: preparation, characteristics and recent advancements. *Cibtech Journal of Bio-protocols*, 1(2): 32-44.
6. Mbaeyi-Nwaoha & Ekere, K. S. (2014). Production and evaluation of flavoured yoghurt from graded levels of soursop (*Annona muricata*) pulp. *Innovare Journal of Food Science*, 2(1): 14-21.
7. FDA. (2013). Yoghurt.21 CFR 131.200, Code of Federal Regulations. U. S. Department of Health and Human Services, Washington, DC.
8. Capozzi, V., Fragasso, M., Romaniello, R., Berbegal, C., Russo, P. & Spano, G. (2017). Spontaneous food fermentations and potential risks for human health. *Fermentation*, 3(4): 1-19.
9. Craig, W. J. & Brothers, C. J. (2021). Nutritional content and health profile of non-dairy plant-based yoghurt alternatives. *Nutrients*, 13: 4069
10. Kent, G., Kehoe, L., Flynn, A. and Walton, J. (2022). Plant-based diets: a review of the definitions and nutritional role in the adult diet. *Proceedings of the Nutrition Society*, 81(1): 62-74.
11. Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M. & Tiwari, B. (2017). Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods*, 6(7), 53
12. Jaeger, S. R., Giacalone, D., Jin, D., Ryan, G. S. & Cardello, A. V. (2023). Information about health and environmental benefits has minimal impact on consumer responses to commercial

plant-based yoghurts. *Food Quality and Preference*, 106: 104820

13. Jeske, S., Zannini, E. & Arendt, E. K. (2018). Past, present and future: The strength of plant-based dairy substitutes based on gluten-free raw materials. *Food Research International*, 110:42-51.
14. Montemurro, M., Pontonio, E., Coda, R. & Rizzello, C. G. (2021). Plant-based alternatives to yoghurt: State-of-the-art and perspectives of new biotechnological challenges. *Foods*, 10 (2), 316.
15. Senadeera, S. S., Prasanna, P. H. P., Jayawardana, N. W. I. A., Gunasekara, D. C. S., Senadeera, P., & Chandrasekara, A. (2018). Antioxidant, physicochemical, microbiological, and sensory properties of probiotic yoghurt incorporated with various *Annona* species pulp. *Heliyon*, 4(11): 1-18.
16. Badrie, N., & Schauss, A. G. (2010). Soursop (*Annona muricata L.*): composition, nutritional value, medicinal uses, and toxicology. In: Bioactive Foods in Promoting Health (Watson, R. R. and Preedy, V. R., Eds.). Academic Press, Cambridge, MA, USA. pp. 621-643.
17. Amadi, J. A. C. & Oly-Alawuba, N. M. (2020). Chemical and sensory properties of soursop smoothie formulated from soursop, tiger nut milk, date palm, and noni juice. *Research Journal of Food and Nutrition*, 4(1): 41-47.
18. AOAC. (2010). Official methods of analysis. Association of Analytical Chemists. 18th Ed. Washington DC.
19. Onwuka, G. I. (2005). Food analysis and instrumentation: theory and practice. Naphthali Prints, Lagos, Nigeria. pp 19-121.
20. Joung, J. Y., Lee, J. Y., Ha, Y. S., Shin, Y. K., Kim, Y., Kim, S. H. & Oh, N. S. (2016). Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts. *Korean Journal for Food Science of Animal Resources*, 36(1): 90-99.
21. Bae, W. Y., Kim, H. Y., Park, E. H., Kim, K. T. & Paik, H. D. (2019). Improved in vitro antioxidant properties and hepatoprotective effects of a fermented *Inula britannica* extract on ethanol-damaged HepG2 cells. *Molecular Biology Reports*, 46(6): 6053–6063.
22. Kim, H. Y., Bae, W. Y., Yu, H. S., Chang, K. H., Hong, Y. H., Lee, N. K. & Paik, H. D. (2020). *Inula britannica* fermented with probiotic *Weissella cibaria* D30 exhibited anti-inflammatory effects and increased viability in RAW 264.7 cells. *Food Science and Biotechnology*, 29(4): 569–578.
23. Clinical & Laboratory Standards Institute (2015). Performance standards for antimicrobial susceptibility testing: 25th informational supplement. CLSI Document M100-S25, Clinical and Laboratory Standards Institute, Wayne, PA
24. Abbo, E. S., Olurin, T. O. & Odeyemi, G. (2006). Studies on the storage stability of soursop (*Annona muricata L.)* juice. *African Journal of Biotechnology*, 5(19): 1808-1812.
25. FDA (2009). Milk and cream products and Yoghurt products. *Food and Drug Administration Federal Register*, 74: 24-48.
26. Ityotagher, A. P. & Julius, A. (2020). Physicochemical composition, sensory properties and keeping quality of functional yoghurt produced from milk-soy flour blends. *Journal of Nutrition, Health and Food Engineering*, 10(1): 5-12.
27. Thompson-Witrick, K. A., Das, K., Choudhary, R., Fisher, D. J. & Chai, T. (2019). Physicochemical and sensory characteristics of made-in-transit yoghurt. *International Journal of Food Science and Nutrition Engineering*, 9(2): 37-44.
28. Mbaeyi-Nwaoha, I. E., Umeh, L. C., Igbokwe, C. J., Obodoechi, C. M. & Okoronkwo, N. C. (2017). Production and evaluation of flavoured yoghurt from graded levels of sweet variety of African bush mango "ugiri" (*Irvingia gabonensis*) juice and pulp. *Food science and Technology*, 5(2): 56-69
29. Virgen-Ceceña, L. J. Anaya-Esparaza, L. M. Corria-Téllez, A. V., García-Magaña, M. d. L., García-Galindo, H. S., Yahia, E. & Montalvo-González, E. (2019). Evaluation of nutritional characteristics and bioactive compounds of soursop-yoghurt and soursop-frozen dessert. *Food Science and Biotechnology*, 28(5): 1337-1347.
30. Dias, P. G. I. & Jayasooriya, M. C. N. (2017). Enhancing the physicochemical and antioxidant properties of stirred yoghurt by incorporating soursop (*Annona muricata*). *International Journal of Life Sciences Research*, 5(1): 69-77.
31. Espinosa, I. A., Basurto, R. I. O., Tovar, B., Mata, M. & Montalvo-González, E. (2013). Physiological and physicochemical behavior of soursop fruits refrigerated with 1-methylcyclopropene. *Journal of Food Quality*, 36(1): 10-20.
32. Akemien, N. N., Adaja, B. O., Simpson, V. B., Akpan, F. S., Khidri, H. Y., & Gumsu, A. A. (2024). Preparation and nutritional evaluation of soursop juice as partial replacement for dairy milk in yoghurt production. *Journal of Applied Science and Environmental Management*, 28(11): 3573-3578.

33. Ani, E., Amove, J. & Igbabul, B. (2018). Physicochemical, microbiological, sensory properties and storage stability of plant-based yoghurt produced from bambara groundnut, soybean and *Moringa oleifera* seed milk. *American Journal of Food and Nutrition*, 6(4):115-125.
34. Nuralifah, Sussilowati, P. E., Parawansah, Ernisa, A., Purnama, L. M. J., Asasutjarit, R.& Fristiohady, A. (2022). Development of probiotic drink from *Dillenia serrata* thumb: antioxidant and antibacterial potencies. *Food Research*, 6(5): 124-129.
35. Su, N., Ren, L., Ye, H., Sui, Y., Li, J. & Ye, M. (2017). Antioxidant activity and flavour compounds of hickory yogurt. *International Journal of Food Properties*, 20(8): 1894-1903.
36. Verni, M., Verado, V. & Rizello, C. G. (2019). How fermentation affects the antioxidant properties of cereals and legumes. *Foods*, 8(9): 362-382.
37. Adebo, O. A. & Medina-Meza, I. G. (2020). Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: a mini review. *Molecules*, 25(4): 927-945.
38. Ning, X., Luo, Z., Chen, Z., Zhou, C., Xie, C., Du, W. & Wang, L. (2021). Fortification of set yoghurt with passion fruit juice: effects onfermentation kinetics, physicochemical properties and functional properties. *Journal of Dairy Science*, 104(4): 4084-4093.
39. Oliveira, A., Alexandre, E. M. C., Coelho, M., Lopes, C., Almeida, D. P. F. & Pintado, M. (2015). Incorporation of strawberry preparation in yoghurt: impact on physicochemical properties and milk proteins. *Food Chemistry*, 171: 370-378.
40. Yurliasni, Novita, C. I., Hanum, Z. & Latif, H. (2020). Optimizing the quality and antimicrobial ability of yoghurt through a combination of starter and date puree at different levels. In IOP Conference Series: *Earth and Environmental Science*, 425(1): 1-4.
41. Aguilar-Hernández, G., López-Romero, B. A., Huerta-Castellanos, I., Téllez-Isaías, G. & Montalvo-González, E. (2024). Acetogenins from *Annona muricata* as antimicrobial agents. In Infectious Diseases. IntechOpen, 115064.
42. Coria-Téllez, A., Montalvo-González, E., Yahia, E. & Obledo-Vázquez, E. (2018). *Annona muricata*: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity.*Arabian Journal of Chemistry*, 11(5): 662-691.
43. Pinto, L. C., Cerqueira-Lima, A. T., Suzarth S. d. S., de Souza, R., Tosta, B. R., da Silva, H. B., Pires, A. O., Queiroz, G. A., Teixeira, T. O., Dourado, K. M. C., Costa, V. O., Baqueiro, V., Oliveira D. P., Brandao, H. R., de Souza, C. O., Drzian, J. I., Medeiros, K. C. P., Alves, C. A. D., Lopes, M. V. & Figueiredo, C. A. V. (2018). *Anonna muricata L.* (soursop) seed oil improves type 1 diabetes parameters *in vivo* and *in vitro*. *PharmaNutrition*, 6(1): 1–8