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# Physicochemical, antioxidant and microbial properties of sweetened yoghurt produced from partial substitution of sugar with soursop puree

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## Abstract

This study was targeted towards evaluating the outcome of partially substituting sugar with soursop puree at different proportions, varying pasteurization temperatures, storage durations and with the application of sodium benzoate and potassium sorbate as chemical preservatives on physiochemical, antioxidant and microbial qualities of yoghurt. There was a strong correlation ( $R^2 = 0.7$ , Adjusted  $R^2 = 0.65$ ) between the syneresis and the water-holding capacity of the yoghurt as the substitution increased. At 50% soursop puree substitution in the yoghurt production, DPPH radical scavenging activity, total phenolic content, vitamin C, carbohydrate, protein, and ash contents increased to 34.40%, 1.25 mg GAE/L, 58.50 mg/100 g, 29.26, 4.02 and 0.69%, respectively. Storage duration does not have a significant influence on the proximate composition of the yoghurt samples. However, the vitamin C content of the yoghurt decreased when the pasteurization temperature was more than 80 °C. The growth of lactic acid bacteria ranged between  $2.9 \times 10^5$  and  $2.16 \times 10^6$  CFU/ml. The use of sodium benzoate inhibited yeast growth more than potassium sorbate while soursop substitution inhibited the growth of mould from  $1.0 \times 10^6$  CFU/ml to  $3.05 \times 10^5$  CFU/ml than the preservatives. Coliform was absent in all the yoghurt samples with or without preservatives. This information would be valuable in yoghurt development and quality control.

**Keywords** Yoghurt, Antioxidant, Preservatives, Microbial, Soursop puree

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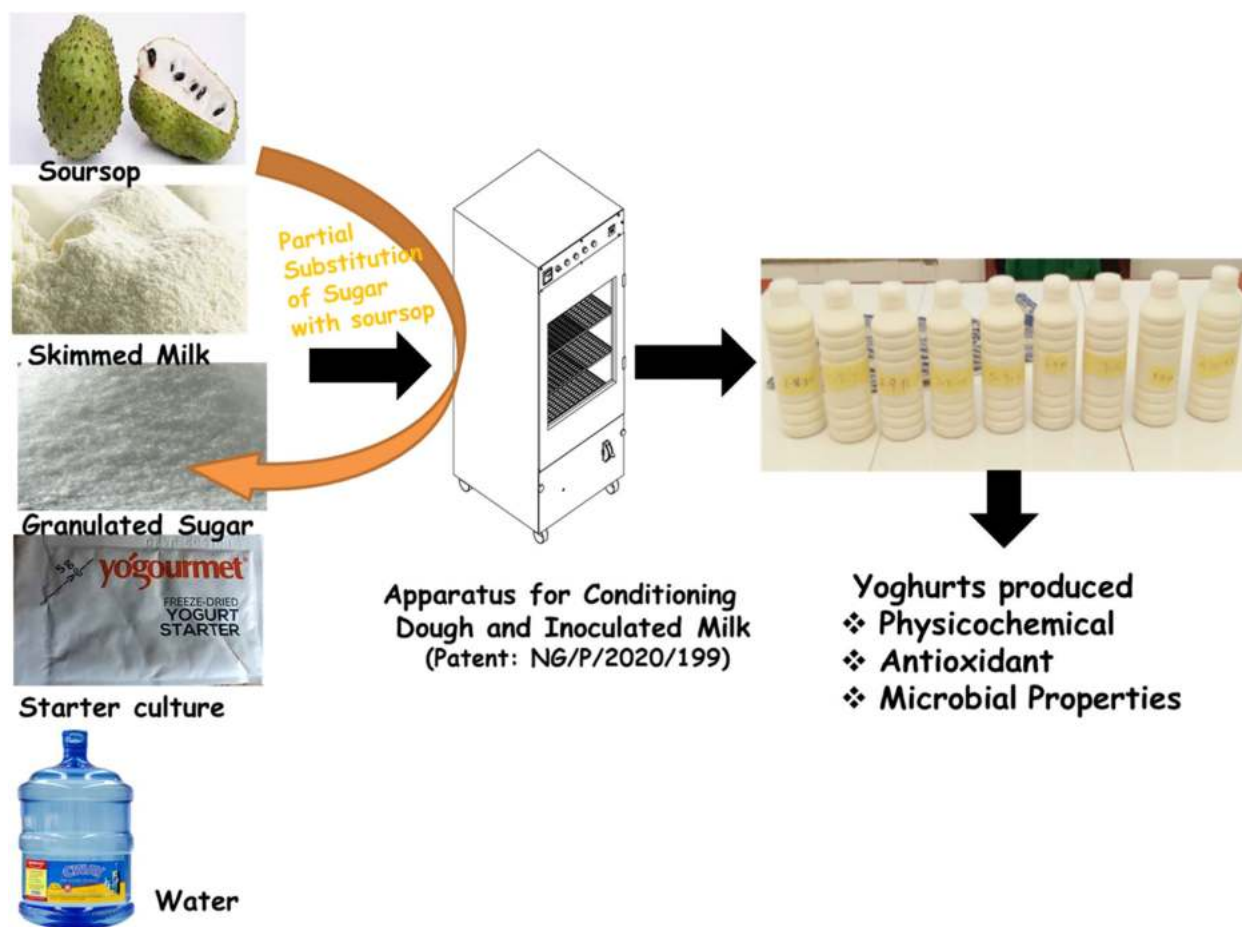
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## Graphical Abstract



## Introduction

For a very long time, fermented drinks and foods contributed greatly to the human diet in terms of their nutritional health benefits (Kanwar & Keshani 2016). Yoghurt is a fermented dairy food product that is greatly enjoyed throughout the world (Fias 2006). In yoghurt production, microorganism cultures like *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are the prominent bacteria associated with yoghurt production (Sanusi et al. 2022). According to Ekere (2014), the tang taste and texture of yoghurt are caused by the action of lactic acid bacteria on milk protein. The presence of lactic acid bacteria in yoghurt also made it a probiotic food product and this helps in enhancing consumers' wellness and good health (Guarner & Schaafsma 1998). Some of the health benefits of yoghurt consumption include increasing the consumer's appetite, arousing

gall secretion, and improving the functionality of the liver and the pancreas (Ndabikunze et al. 2017). It was reported by Medeiros et al. (2015) that the temperature of pasteurization, the composition of milk and the temperature of incubation are the determinants that influence the physiochemical attributes of yoghurt during its production. Yoghurt is highly susceptible to spoilage because it contains a very high amount of nutrients which makes it undergo swift and unpleasant chemical and microbial changes which hinder its shelf life. As a result of this, during the production of yoghurt, preservatives are sometimes added by some producers. Sorbic and benzoic acids including their salts-potassium sorbate ( $C_6H_7KO_2$ ) and sodium benzoate ( $C_7H_5NaO_2$ ) are widely used food preservatives because they are responsible for lowering the pH of food (Amirpour et al. 2015). Teshome et al. (2017) also reported that potassium sorbate and sodium benzoate are useful

in preventing the growth and multiplication of varieties of bacteria, yeast and mould. For preservation, Mroueh et al. (2008) reported that sodium benzoate and potassium sorbate could be used in the range of 0.05–0.1% and 0.02–0.3% respectively.

In the last decade, health-beneficial ingredients like vegetables and fruits have been added to yoghurt to improve its sensory, nutritional, and functional characteristics. A research study carried out by Ihemeje et al. (2015) showed an increase in nutritional and flavour contents of yoghurt fortified with pineapple, carrot, pepper, and ginger when compared with plain yoghurt. Additionally, it was observed that the addition of papaya and mango juice to yoghurt largely increases its physiochemical and sensory attributes (Teshome et al. 2017). *Annona muricata* L. is a juicy, acidic, and aromatic tropical fruit with many therapeutic and nutritive properties (Senadeera et al. 2018). Most English-speaking countries usually referred *Annona muricata* L. as Soursop while in some countries like Germany, Malaysia, Netherlands, and Venezuela, they are called Sauersak, Durian belanda, Zunrzak and Catoche (Badrie & Schauss 2010). Soursop originated from the Caribbean or Southern America and is currently distributed in the tropical and subtropical regions of the world (Prance 2003). Mexico is the largest producer and consumer of soursop (Coelho & Alves 2011). The whitish edible pulp of Soursop contains water, titratable acidity, carbohydrate, protein and non-reducing sugar of 80, 3.43, 18, 1 and 24.5%, respectively (Badrie & Schauss 2010). Soursop pulp is also a rich source of vitamins B<sub>1</sub>, B<sub>2</sub>, and C and antioxidants such as total phenols, flavonoids, and anthraquinones (Badrie & Schauss 2010; Rubio-Melgarejo et al. 2020).

In West African countries, most especially in Nigeria, soursop is an underutilized tropical fruit. Nevertheless, certain people who are conscious of their health and taking measures against health challenges such as diabetes and obesity are now decreasing the rate at which they consume sugar by taking products which could be sweetened with aromatic natural fruit like soursop puree as this could improve the nutrient content of yoghurt. Some researchers have determined the functional, organoleptic, and nutritional properties of yoghurt produced with soursop. For instance, a study that uses different types of soursops in the production of yoghurt was carried out by Senadeera et al. (2018). Saputrayadi et al. (2021) also reported that the sensory attributes and antioxidant activity of yoghurt increased when soursop was added. Also, the addition of soursop fruit extract to sweetcorn milk yoghurt improved the lactic acid levels, organoleptic attributes and vitamin C of the yoghurt (Saputrayadi et al. 2021). A profile that explained the

kinetic acidification of yoghurt using soursop was reported by Sanusi et al. (2022). However, there is limited information on the interactive effect of partial substitution of sugar with soursop, varying pasteurization temperatures, storage durations and the application of sodium benzoate and potassium sorbate on physiochemical, antioxidant and microbial qualities of yoghurt. The above information will be beneficial for determining the required conditions that would retain nutrients. Also, it gives insights into the use of food preservatives on the shelf life of yoghurt when the sugar is partially substituted with soursop puree. Therefore, the objective of the study was to evaluate the effects of partially substituting sugar with soursop puree, usage of chemical preservatives, storage time and varying pasteurization temperature on the antioxidant, physiochemical and microbial characteristics of yoghurt.

## Methodology

### Materials

The *Annona muricata* L. used in this study was obtained from Ilorin, Nigeria. The specific lactic acid bacteria starter culture that was used was a combination of freeze-dried *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The lactic acid bacteria culture was obtained from KetoNaija, Lagos, Nigeria. The skimmed milk powder and sugar incorporated to produce this yoghurt were obtained from Arla Foods (Dano Milk) Lagos, Nigeria and Dangote Sugar, Nigeria while the preservatives (potassium sorbate and sodium benzoate) were purchased from Lagos Nigeria at Azchem LTD.

### Soursop puree preparation

Fully mature and ripe soursop was washed with potable water, stripped of its peels and the seeds were removed. The soursop puree was made by blending the pulp in an electric blender (Model: SCB-505, China) until a fine and smooth puree was obtained. It was then packed and stored in a refrigerator until required for use.

### Taguchi experimental design

The experiment was designed using Taguchi orthogonal array L<sub>9</sub><sup>(3<sup>\*\*\*4</sup>)</sup> via a statistical software known as Minitab 16. The range of process treatments for yoghurt production using the Taguchi design is presented in Table 1. This experimental design was used to determine the effects of partially substituting sugar with soursop puree, varying pasteurization temperature, chemical preservatives and storage time on the microbial, physiochemical and antioxidant attributes of yoghurt samples. Nine (9) samples of yoghurt were obtained from the experimental design.

**Table 1** Experimental design of interactive process treatment for yoghurt production

| Process treatment                                    | Code | Unit | Rank 1  | Rank 2 | Rank 3 |
|------------------------------------------------------|------|------|---------|--------|--------|
| Pasteurization temperature (°C)                      | PT   | °C   | 80      | 90     | 100    |
| Partial substitution of sugar with soursop puree (%) | PSSS | %    | 0       | 25     | 50     |
| Chemical Preservative                                | CP   |      | Control | SB     | PS     |
| Storage duration                                     | SD   | days | 7       | 14     | 28     |

Where SB is the sodium benzoate, PS is the potassium sorbate and control (no soursop and 100% sugar)

### Production of yoghurt

The modified steps described by Sanusi et al. (2022) were employed in the production of the yoghurt. The ingredients used for the plain yoghurt were accurately measured using an electric measuring scale (Model: Camry, China). The following were measured; 10,000l of water, 1500g of skimmed milk, 800g of sugar and 5g of freeze-dried starter culture. Substituting sugar partially with soursop was done by varying their quantities as depicted in Table 1. Pasteurization temperatures (80–100°C) were used to dissolve the skimmed milk, soursop puree and sugar. Afterwards, it was fully homogenized using a mixer to get a uniform mixture. The fully homogenized product was then left to a lukewarm temperature of 45°C, which is a suitable inoculation temperature for the proliferation of the lactic acid bacteria. Polypropylene bottles were used to store the inoculated samples before transferring them into a patented instrument meant for inoculating milk and dough conditioning as shown in Fig. 1. All the inoculated samples were then incubated at  $40 \pm 2^\circ\text{C}$  and the pH was checked after 5 h of incubation and at 30 min intervals

until a pH of 4.6 was achieved. The pH was measured using a digital pen-style pH meter (Model: PH-W2, India) after it has been calibrated with a buffer solution. Potassium sorbate and sodium benzoate were separately added to the total mass of the yoghurt at 0.02 and 0.05%, respectively. The produced sweetened stirred yoghurt samples obtained were kept at a refrigerated condition of 4°C and stored for 7–28 days as indicated in Table 1. Figure 2 shows the flow chart for sweetened yoghurt produced from the partial substitution of sugar with soursop puree.

### Physicochemical attributes

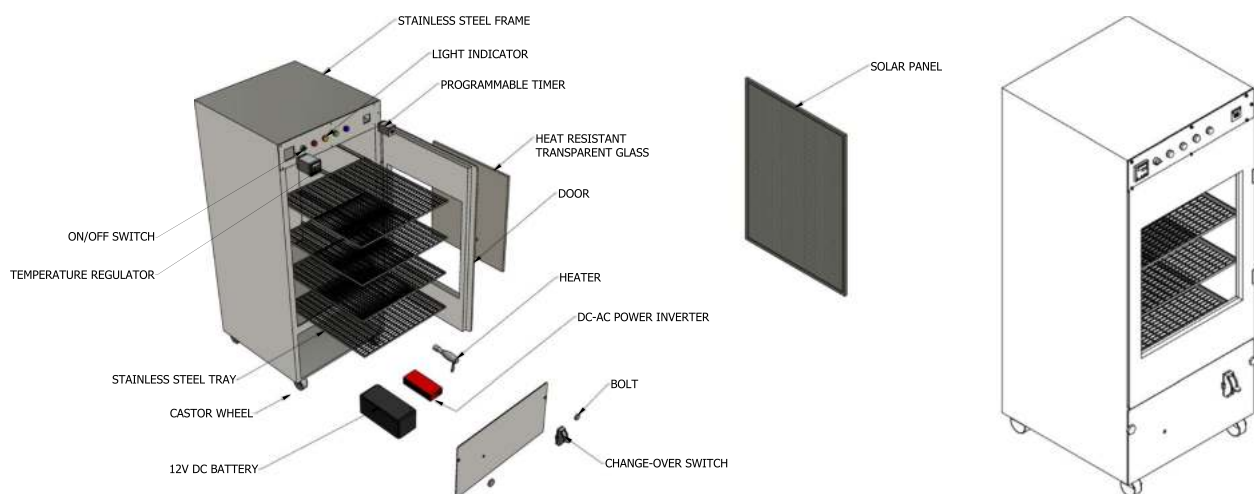
#### Syneresis

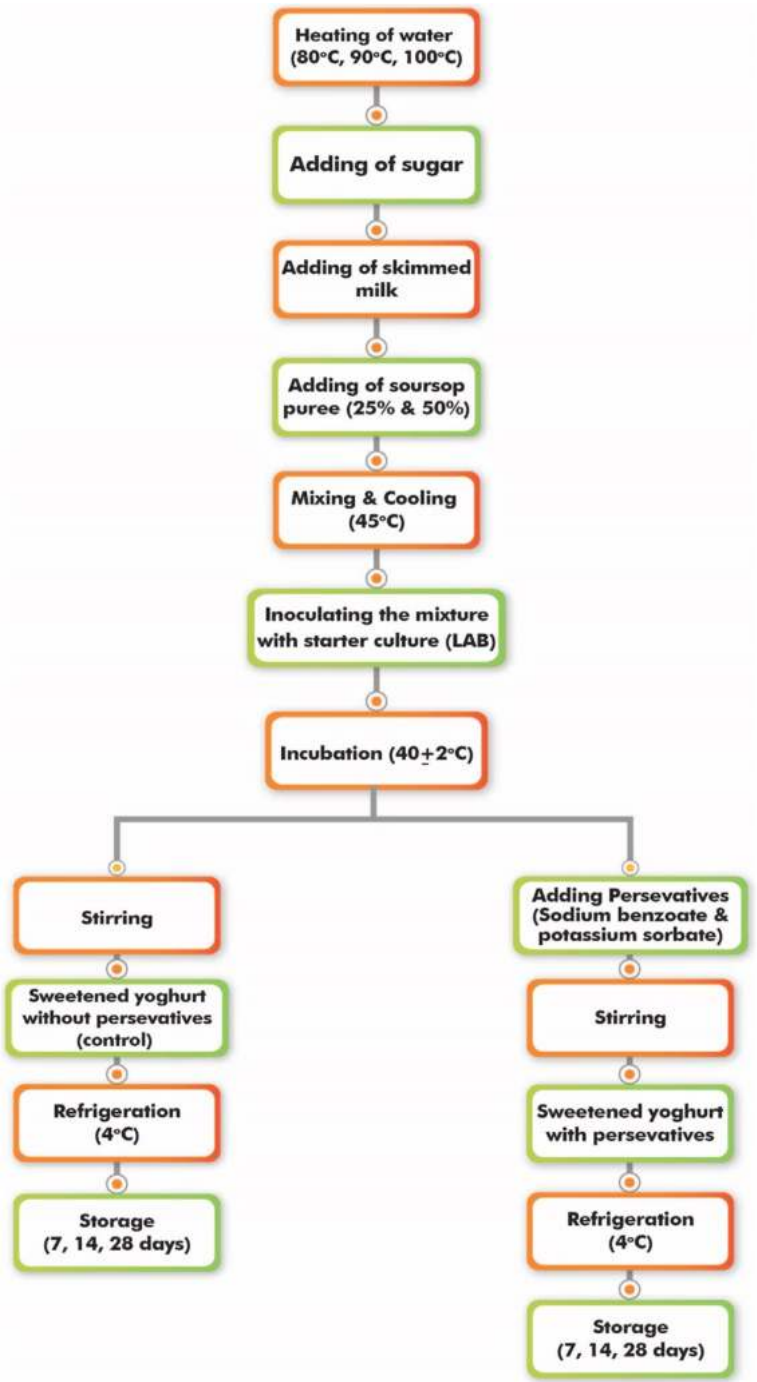
A modified centrifugation method as stated by Joung et al. (2016) was used to evaluate the syneresis (%) of the yoghurt samples. After the target duration of storage has been reached, the syneresis was evaluated by centrifuging 20g of yoghurt sample in 50 ml glass tubes at 3500rpm at 20°C for 15 min. Equation 1 was used to estimate the syneresis.

$$\text{Syneresis (\%)} = \left( \frac{\text{weight of supernatant}}{\text{weight of yoghurt sample}} \right) \times 100 \quad (1)$$

#### Water holding capacity

The modified method as stated by Guzmán-González et al. (1999) was used to determine the water-holding capacity of the yoghurt samples. An approximate 20 g yoghurt sample was centrifuged at  $1250 \times g$  at 40°C for 10 mins. The whey obtained after the yoghurt sample has undergone centrifugation was collected and measured. Equation 2 was used to determine the water-holding capacity of the yoghurt sample ( $\text{WHC, g.kg}^{-1}$ )

**Fig. 1** Patented device for conditioning inoculated milk and dough (Sanusi & Sumonu 2020)



**Fig. 2** Flow chart for sweetened yoghurt produced from the partial substitution of sugar with soursop puree

$$WHC = \frac{Y - W}{Y} \times 100$$
 (2)

where, Y is the mass of the yoghurt sample and W is the mass of the whey obtained.

**Proximate composition**  
The proximate composition of the yoghurt samples was evaluated using the AOAC (2010) and the carbohydrate was determined by a deduction method.

### Antioxidant properties

#### Vitamin C determination

The titrimetric procedure described by Joy and Saumya (2013) was employed in the determination of the vitamin C content of the yoghurt samples. Equation 3 was used to calculate the vitamin C content of the yoghurt samples.

$$\text{Vit.C content (mg/100g)} = \frac{\text{Titre value} \times \text{dye factor} \times \text{total volume made} \times 100}{\text{weight of samples} \times \text{aliquot taken}} \quad (3)$$

#### Total phenolic content (TPC) determination

The modified method employed by Zhou et al. (2004) was used to determine the TPC. Eight hundred microliters of 10% Na<sub>2</sub>CO<sub>3</sub> and 400 uL of 2% 2N Folin-Ciocalteu's phenol reagent were both added and mixed thoroughly with a 200 uL precisely diluted yoghurt sample. It was then kept in an incubator for 3 mins. Afterwards, the mixture was left for 1 h and covered with an aluminum foil at room temperature (25 ± 2 °C) without exposure to light. The absorbance of the mixture was determined at 750 nm using a microplate reader while it was made to undergo vorticity. Gallic acid was used to obtain a standard curve and the measurements were specified in milligrams of gallic acid equivalent per litre (mg GAE/L).

#### DPPH (2,2 Diphenyl-1-picrylhydrazyl) radical-scavenging activities determination

Zhang et al. (2018) method of DPPH determination was used. Twenty milliliters aliquots were amassed from the yoghurt samples and were centrifuged at 9000 rpm<sup>-1</sup> for 20 mins and the supernatant was filtered on a 0.45 µm filter. The DPPH reagent of 0.01 mM and yoghurt supernatant were homogenized in a plate of 96-well. It was left at room temperature (25 ± 2 °C) without exposure to light in order to react well for 30 mins. Std curve range was obtained via the gallic acid, and the measurements were dimensioned in milligrams of gallic acid equivalent per litre (mg GAE/L). Equation 4 was used to determine the DPPH scavenging activity.

$$\text{DPPH Scavenging (\%)} = \left[ 1 - \left( \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \right] \times 100\% \quad (4)$$

where Abs means the absorbance read at 515 nm.

### Microbial analysis

#### Lactic acid bacteria (LAB) enumeration

The Miles-Misra procedure reported by Baron et al. (2006) was used to enumerate the lactic acid bacteria present in the samples. A sterile normal saline was used in

the preparation of the dilution whereby MRS and M17 agar media were used to culture the lactic acid bacteria at an incubation temperature of 48 °C and 42 °C, respectively. It was exposed to 10% carbon dioxide for a period of 48 h.

#### Enumeration of coliform

Coliform was evaluated using Hervert et al. (2017) method. It was determined by mixing a sterile MacConkey Agar with 1 ml of yoghurt dilution 10–1 inside a Petri dish after which it was left to set. It was then added to another layer of MacConkey agar and placed in an incubator for a period of 24–28 h at 37 °C.

#### Enumeration of mould and yeast

Taniwaki et al. (2001) method of mould and yeast enumeration was used. It was carried out by using a sterile potato dextrose agar as a medium of culture, a pasteur pipette and 1 ml of 10<sup>-1</sup> diluted yoghurt sample (1 ml) which was dissipated on the surface. The plates used were placed in an incubator for 5–7 days at 25 °C.

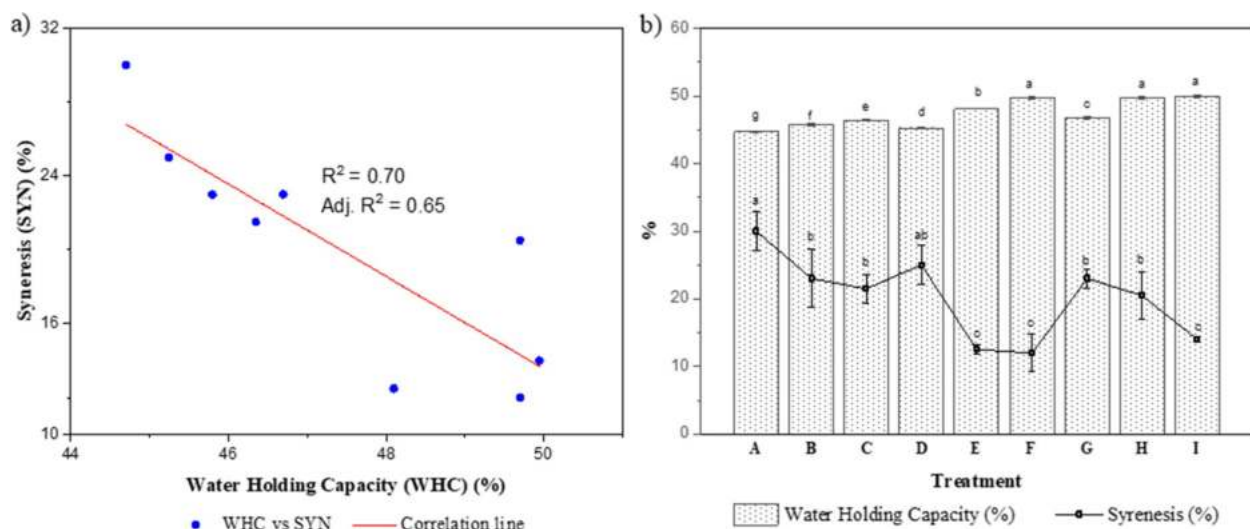
#### Statistical analysis

The experiments were run in duplicates and the obtained data were analyzed statistically at 95% confidence levels using Duncan's new multiple range test (DNMRT) of one-way analysis of variance (ANOVA) in Statistical Package for Social Science (SPSS) version 26.

## Results and discussion

#### Yoghurt Syneresis and water holding capacity

The effect of process treatment showed that a strong correlation was noted ( $R^2 = 0.7$ , Adjusted  $R^2 = 0.65$ ) between the syneresis and water-holding capacity of the yoghurt samples (Fig. 3a). The effect of partially substituting sugar with soursop puree, the temperature of pasteurization, chemical preservatives, and duration of storage on the water holding capacity and syneresis of yoghurt is presented in Fig. 3b. The mean and standard deviation of the syneresis and water holding capacity with different superscript letters along the same treatment are significantly different at  $p \leq 0.05$  (Fig. 3b). The water holding capacity of the yoghurt samples ranged between  $44.70 \pm 0.14$  and  $49.95 \pm 0.21\%$  while syneresis ranged between  $12.00 \pm 2.83$  and  $30.00 \pm 2.83\%$ . The lowest syneresis value was recorded in treatment F ( $12.00 \pm 2.83$ ) at 50% soursop puree substitution, temperature of pasteurization

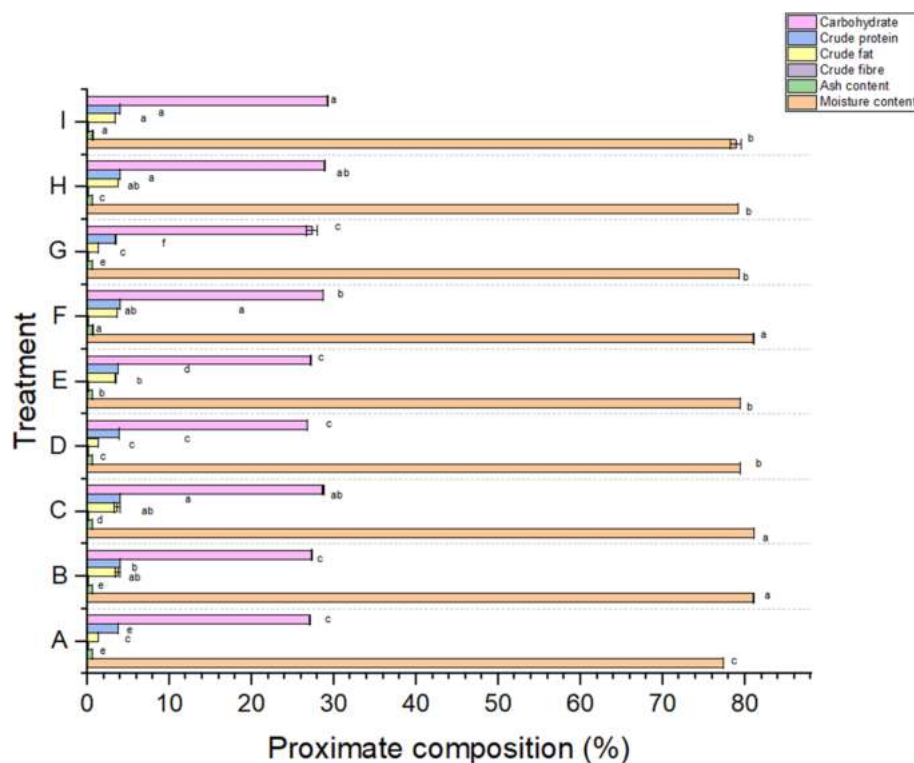


**Fig. 3** a Correlation of syneresis and water holding capacity of the yoghurt samples (b) Effect of process treatment on syneresis and WHC of the yoghurt samples. Note: Treatments: A (control) is 80°C PT, 0% SSP, No CP and 7 days SD; B is 80°C PT, 25% SSP, sodium benzoate CP and 14 days SD; C is 80°C PT, 50% SSP, potassium sorbate CP and 28 days SD; D is 90°C PT, 0% SSP, sodium benzoate CP and 28 days SD; E is 90°C PT, 25% SSP, potassium sorbate CP and 7 days SD; F is 90°C PT, 50% SSP, No CP and 14 days SD; G is 100°C PT, 0% SSP, potassium sorbate CP and 14 days SD; H is 100°C PT, 25% SSP, No CP and 28 days SD; I is 100°C PT, 50% SSP, sodium benzoate CP and 7 days SD

at 80°C, potassium sorbate used, and storage duration of 28 days. The highest water holding capacity value was recorded in treatment I ( $49.95 \pm 0.21$ ) at a pasteurization temperature of 100°C, 50% soursop puree substitution, sodium benzoate was used as a chemical preservative and the duration of storage was 7 days. Although, no significant difference at  $p > 0.05$  was observed in the water-holding capacity of treatments F and I. However, the lowest water holding capacity ( $44.70 \pm 0.14\%$ ) and highest syneresis value ( $30.00 \pm 2.83\%$ ) were observed in treatment A which serve as a control or plain yoghurt and processed at pasteurization temperature of 80°C and 7 days storage duration. Lower syneresis values were observed in the yoghurt with process treatments E, F and I and this could be traced to the partial substitution of sugar with soursop puree. The partial substitution of sugar with soursop puree that occurred in treatment I and did not take place in treatment A made them significantly different ( $p < 0.05$ ). These findings showed that partially substituting sugar with soursop puree could decrease the syneresis and increase the apparent viscosity of yoghurt. In the work of Mohammadi-Gouraji et al. (2018), it was reported that the syneresis of plain yoghurt was higher while the one enriched with phycocyanin was lower. Also, yoghurt samples enriched with *moringa oleifera* extract had higher water holding capacity and lower syneresis values (Zhang et al. 2018). Thus, it can be said that raising the total solid content of yoghurt results in a more stable gel matrix (yoghurt curd) with higher water holding capacity and reduced syneresis.

### Proximate composition of yoghurt

Figure 4 shows the proximate composition of the nine yoghurt samples processed under different treatments. In Fig. 4, the mean and standard deviation of the composition with different superscript letters along the same treatment are significantly different at  $p \leq 0.05$ . The carbohydrate ranged between 26.77 and 29.26%, crude protein (3.47 and 4.02%), moisture content (77.40 and 81.09%), crude fibre content (0.10 and 0.12%), ash (0.59 and 0.69%) and fat (1.40 and 3.81%), respectively. Pasteurization temperature below 100°C increased the moisture content of the yoghurt samples when sugar was partially substituted with soursop puree. Although, at a pasteurization temperature of 100°C and when soursop puree was included, the moisture content of the yoghurt samples decreased. This could be attributed to the effect of evaporation at 100°C. The partial substitution of sugar with soursop puree and preservatives also increased the ash content of the yoghurt samples. This could be due to the presence of minerals in the soursop puree and preservatives used. Mbaeyi-Nwaoha and Nwachukwu (2012), also observed an increase in the ash content of yoghurt flavoured with beetroot. The yoghurt produced at different treatment levels did not significantly affect the crude fibre as there was no significant difference at  $p > 0.05$ . This corroborates with the findings of Matela et al. (2019) that yoghurts have very low fibre content, and this could be attributed to the fact that it is milk and water-based products. The fibre content obtained in this study is higher than 0.01 to 0.07%



**Fig. 4** Effect of process treatment on the proximate composition of yoghurt. Note: Treatments: **A** (Control) is 80 °C PT, 0% SSP, No CP and 7 days SD; **B** is 80 °C PT, 25% SSP, sodium benzoate CP and 14 days SD; **C** is 80 °C PT, 50% SSP, potassium sorbate CP and 28 days SD; **D** is 90 °C PT, 0% SSP, sodium benzoate CP and 28 days SD; **E** is 90 °C PT, 25% SSP, potassium sorbate CP and 7 days SD; **F** is 90 °C PT, 50% SSP, No CP and 14 days SD; **G** is 100 °C PT, 0% SSP, potassium sorbate CP and 14 days SD; **H** is 100 °C PT, 25% SSP, No CP and 28 days SD; **I** is 100 °C PT, 50% SSP, sodium benzoate CP and 7 days SD

reported for some yoghurt samples in Maseru, Lesotho by Matela et al. (2019). The yoghurts with the highest crude fat content (3.81%) were observed in treatments B, C, F and H with no significant difference at  $p \geq 0.05$ . The increase in the crude fat content occurred in yoghurt with 25 and 50% partial substitution of sugar with soursop puree while the yoghurt with the lowest crude fat content was observed in the control that has no soursop puree and preservatives. Fat content plays an important role in yoghurts as it improves the flavour, taste, appearance, and texture of yoghurts (Oladipo et al. 2014). The yoghurts with 25 and 50% partial substitution of sugar with soursop puree could be classified as regular yoghurt because its fat content is more than 3.25% while the control yoghurt could be categorized as low fat because the fat content is between 0.5–2%. This classification is in line with United States Department of Agriculture (USDA) (2001) specifications for yoghurt, non-fat yoghurt and low-fat yoghurt. The yoghurt protein content increases with an increase in the level of soursop puree added. The increase in the protein content could be attributed to the protein content present in the soursop pulp in addition to the high protein

content of milk. Enweani et al. (2004) reported a protein content of 2.91% in soursop. On the other hand, animal milk has a high protein quality as it provides all the amino acids the body needs to function efficiently. The protein obtained in this study is higher than the 4.01% reported by Yousef et al. (2013) in the production of apple yoghurt. In addition, the protein content obtained in this study is above the 2.7% minimum requirement for yoghurt reported by CODEX (2018). The carbohydrate slightly increased with 50% partial substitution of sugar with soursop puree. The highest value (29.26%) of carbohydrate obtained is higher than those reported by Ibhaze et al. (2021) with 11.86% carbohydrate content in grape flavoured yoghurt. This could be attributed to the difference in carbohydrate content of grape and soursop fruit used in production. The storage time had an indistinguishable effect on the proximate composition of the yoghurt samples.

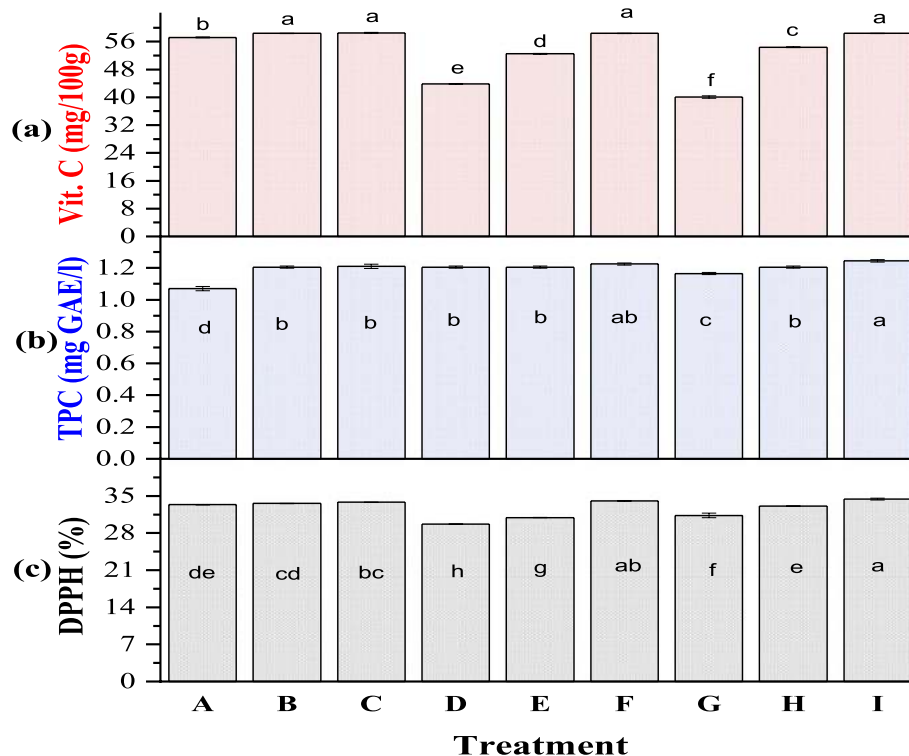
#### Effect of process treatment on yoghurt vitamin C content

Figure 5a presented the effect of process treatments on the vitamin C content of the yoghurt samples. The mean and standard deviation of the vitamin C with different

superscript letters along the same treatment are significantly different at  $p \leq 0.05$  (Fig. 5a). The vitamin C content ranged between  $40.05 \pm 0.35$  mg/100g and  $58.50 \pm 0.07$  mg/100g. The vitamin C content increased when sugar was partially substituted with soursop puree which could be attributed to the high content of vitamin C in the soursop fruits. According to Badrie and Schauss (2010), soursop as a vitamin C content of 29.6 mg/100g. However, it was reduced when the samples were subjected to a pasteurization temperature of above  $80^\circ\text{C}$  and this could be attributed to the degradation of vitamin C at a higher temperature. Uckiah et al. (2009) stated that treating pineapple at a temperature of  $90^\circ\text{C}$  reduced its vitamin C content. The addition of preservatives to the yoghurt samples at a pasteurization temperature of  $80^\circ\text{C}$  slightly increased the vitamin C when compared to plain yoghurt without preservatives. Although, the type of preservative that was more effective could not be distinguished. The duration of storage did not have much effect on the vitamin C of the yoghurt samples which could be attributed to the storage temperature of  $4^\circ\text{C}$  used.

#### Effect of process treatment on yoghurt total phenolic content and DPPH radical scavenging activity

Figure 5b shows the total phenolic content of the yoghurt samples. The mean and standard deviation of the total phenolic content with different superscript letters along the same treatment are significantly different at  $p \leq 0.05$  (Fig. 5b). It ranged from  $1.07 \pm 0.01$  to  $1.25 \pm 0.00$  mg GAE/L. The samples partially substituted with soursop puree had higher total phenolic content compared to plain yoghurt, and this could be a result of the phenolic content present in soursop fruit. In a food sample, the bioactive components can be pointed out with the presence of antioxidants and an example of such is DPPH radical scavenging activity. According to Rafieian-Kopaei et al. (2013), degenerative diseases that cause damage to the cells and tissues could be inactivated by consuming antioxidant-rich food. Figure 5c showed the outcome of DPPH radical scavenging activity levels in yoghurt samples under different treatments. The mean and standard deviation of the DPPH with different superscript letters along the same treatment are significantly different

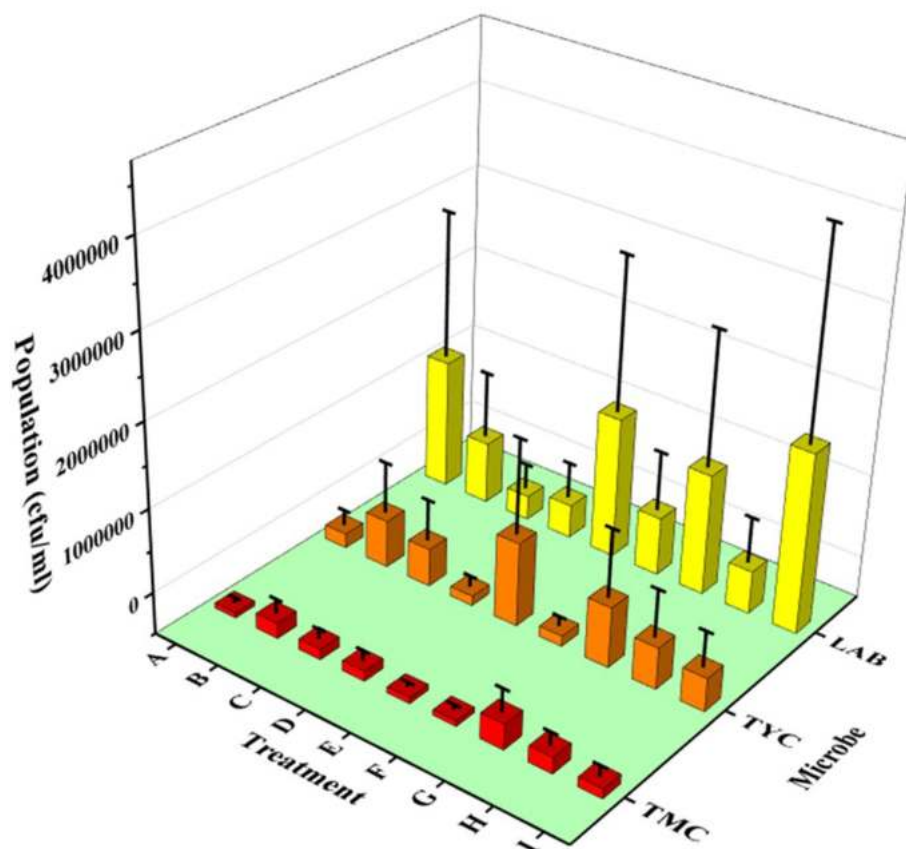


**Fig. 5** Effects of pasteurization temperature (PT), partial substitution of sugar with soursop puree, preservatives (PV) and storage duration (SD) on (a) vitamin C content, (b) total phenolic content (TPC) and (c) 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of yoghurt samples. Note: Treatments: A is  $80^\circ\text{C}$  PT, 0% SSP, No CP and 7 days SD; B is  $80^\circ\text{C}$  PT, 25% SSP, sodium benzoate CP and 14 days SD; C is  $80^\circ\text{C}$  PT, 50% SSP, potassium sorbate CP and 28 days SD; D is  $90^\circ\text{C}$  PT, 0% SSP, sodium benzoate CP and 28 days SD; E is  $90^\circ\text{C}$  PT, 25% SSP, potassium sorbate CP and 7 days SD; F is  $90^\circ\text{C}$  PT, 50% SSP, No CP and 14 days SD; G is  $100^\circ\text{C}$  PT, 0% SSP, potassium sorbate CP and 14 days SD; H is  $100^\circ\text{C}$  PT, 25% SSP, No CP and 28 days SD; I is  $100^\circ\text{C}$  PT, 50% SSP, sodium benzoate CP and 7 days SD

at  $p \leq 0.05$  (Fig. 5c). The values observed ranged between  $29.68 \pm 0.01$  and  $34.40 \pm 0.19\%$  and the lowest value was observed in treatment D which was a plain yoghurt that was treated at  $90^\circ\text{C}$  pasteurization temperature and stored for 28 days while sodium benzoate was used as a chemical preservative. The treatment I had the highest DPPH at a pasteurization temperature of  $100^\circ\text{C}$  and substituted with 50% soursop puree at 7 days and with the use of sodium benzoate. At  $p < 0.05$ , the DPPH radical scavenging activity levels of the treated yoghurt which was substituted with soursop puree were significantly high. This could be as a result of the presence of phenolic content and vitamin C in the soursop fruits. It also showed that the soursop puree incorporated in the yoghurt will enable it to have better health-benefitting attributes and higher antioxidant properties. Nguyen and Hwang (2016) reported that yoghurt incorporated with Aronia (*Aronia melanocarpa*) had higher antioxidant properties than plain yoghurt.

#### Effect of process treatment on yoghurt microbial properties

Figure 6 presented the effect of process treatment on the total yeast, total mould and lactic acid bacteria counts of the yoghurt samples. The results showed the absence of coliform counts in the yoghurts. The sanitary conditions used in producing the products and the storage temperature of  $4^\circ\text{C}$  used in keeping the products could be the reason for the absence of coliform. No coliform count is a suggestion of products of acceptability (CODEX 2018). The growth of lactic acid bacteria ranged between  $2.9 \times 10^5$  CFU/ml and  $2.16 \times 10^6$  CFU/ml with storage duration influencing it. This is evident in treatment I which is 7 days and with the highest lactic acid bacteria as against treatment C which is 28 days with the lowest lactic acid bacteria presence. This outcome established an inverse relationship between storage duration and lactic acid bacteria growth. Therefore, freshly produced yoghurt contains more active lactic acid



**Fig. 6** Effect process treatments on lactic acid bacteria, total mould and total yeast counts of the yoghurt samples. Note: Treatments: A is  $80^\circ\text{C}$  PT, 0% SSP, No CP and 7 days SD; B is  $80^\circ\text{C}$  PT, 25% SSP, sodium benzoate CP and 14 days SD; C is  $80^\circ\text{C}$  PT, 50% SSP, potassium sorbate CP and 28 days SD; D is  $90^\circ\text{C}$  PT, 0% SSP, sodium benzoate CP and 28 days SD; E is  $90^\circ\text{C}$  PT, 25% SSP, potassium sorbate CP and 7 days SD; F is  $90^\circ\text{C}$  PT, 50% SSP, No CP and 14 days SD; G is  $100^\circ\text{C}$  PT, 0% SSP, potassium sorbate CP and 14 days SD; H is  $100^\circ\text{C}$  PT, 25% SSP, No CP and 28 days SD; I is  $100^\circ\text{C}$  PT, 50% SSP, sodium benzoate CP and 7 days SD

bacteria than aged yoghurt. For the mould counts, the results showed that they ranged from  $3.05 \times 10^5$  to  $6.5 \times 10^4$  CFU/ml. The preservative does not have much effect on the total mould counts compared with the proportion of the partial substitution of soursop to sugar. This could be deduced from the result of treatment G (plain yoghurt), which had the highest mould count while treatment F produced with 50% substitution had the lowest mould count. In the absence of partial substitution of sugar with soursop, yoghurt produced with sodium benzoate exhibited a higher inhibiting effect than the ones produced with potassium sorbate. Therefore, incorporating soursop puree in yoghurt without the use of chemical preservatives can reduce mould counts regardless of the storage duration. The citric acid and malic acid which are organic acids of soursop could be attributed to the reduction of mould counts. Higgins and Brinkhaus (1999), reported that organic acids are good inhibitors of mould. The total yeast counts ranged between  $1.0 \times 10^6$  and  $1.2 \times 10^5$  CFU/ml. It was observed that the pasteurization temperature, the substitution of sugar with soursop and storage duration does not have a major effect on yeast counts but the preservative does as observed in treatment E and D. Potassium sorbate showed a less inhibiting effect on yeast growth than sodium benzoate.

## Conclusions

This study established that partially substituting sugar with soursop puree elevates the water-holding capacity and reduces the syneresis of the yoghurt samples. The partial substitution of sugar with 50% soursop puree led to an increase in the carbohydrate, crude fat, vitamin C, crude protein, DPPH radical scavenging activity and total phenolic content of the yoghurt. There was a reduction in the vitamin C content of the yoghurt at pasteurization temperature of above  $80^\circ\text{C}$  while coliform was absent in the yoghurt samples. The incorporation of sodium benzoate as a chemical preservative prevents the growth of yeast while the presence of soursop puree in the yoghurt was found to inhibit the mould more than the preservative. Furthermore, shorter storage durations increase the lactic acid bacteria counts when compared with longer duration of storage. Lastly, this study would be of beneficial value to yoghurt manufacturers in the aspect of product development and it can be suggested that the benefits of soursop could be more easily accessible if incorporated into yoghurt which is more widely consumed.

## Supplementary Information

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**Additional file 1: Table 1.** Soursop composition.

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## Authors' contributions

Sanusi, M.S. conceived the idea, designed the experiments, analyzed the data of the experiment, and drafted the revised manuscript. Sunmonu, M.O. and Alaka A. reviewed the manuscript and analysed the data. Raji A. reviewed and edited the manuscript. Abdulazeez A., Victoria J. and Adeyemi I. helped to collect the data and draft the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

All data supporting this study are included in this manuscript. Further details are available from the corresponding author upon request.

## Declarations

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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