Impacts of Pasteurisation Temperature, Preservatives, Storage Duration and Soursop Puree Substitution for Sugar on Physicochemical, Antioxidant and Microbial Properties of Set-Type Yoghurt

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Abstract

This study aimed to evaluate the impacts of pasteurization temperature (80°C, 90°C and 100°C), soursop puree substitution for sugar (0%, 25%, and 50%), storage duration (7, 14 and 28 days) and preservatives (potassium sorbate, sodium benzoate and no preservative) on the physicochemical, antioxidant and microbial properties of set-type yoghurt. The water holding capacity and syneresis of the yoghurt varied as the soursop puree to sugar ratio increased. Replacement of sugar with 50% soursop puree increased ash, protein, carbohydrate, vitamin C, total phenolic content and DPPH of the yoghurt to 0.69%, 4.02%, 29.26%, 58.50%, 1.25 mg GAE/l and 34.40%, respectively and no coliform was detected. Lactic acid bacteria counts decreased as the storage duration increased while sodium benzoate inhibits mould and yeast counts more than the potassium sorbate. The soursop puree substitution with up to 50% of sugar could be used to produce yoghurt with potential health benefits and increased shelf life.

1. Introduction

Since ancient times, fermented foods and beverages have been a vital part of the human diet, providing significant health benefits (Ansorena & Artiasaran, 2016; Kanwar & Keshani, 2016). Yoghurt, a Turkish word referring to milk curdled with lactic acid bacteria (LAB) or probiotic starter culture, is a fermented dairy product widely consumed globally (Fias, 2006). *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus salivarius subsp. thermophiles* and *Lactobacilli bifidobacteria* are the prominent LAB used in yoghurt production. The fermentation of milk protein by the LAB is responsible for yoghurt's texture and its characteristic tang taste (Ekere, 2014). The presence of live active LAB in yoghurt also makes it a probiotic product, promoting consumers' good health and well-being (Guarner & Shaafsma, 1998). According to Ndabikunze et al. (2017), consuming yoghurt could help increase one's appetite, stimulate bile secretion, and enhance the functions of the pancreas and the liver. In yoghurt production, milk composition, pasteurization treatment and the incubation temperature had been reported as some of the factors that could influence the physicochemical properties of yoghurt (Medeiros et al., 2015). Yoghurt has high nutrient density and may undergo rapid undesirable microbial or chemical changes which could shorten its shelf-life. Consequently, preservatives are often included in yoghurt production. Benzoic and sorbic acids and their respective salts - sodium benzoate ($C_7H_5NaO_2$) and potassium sorbate ($C_6H_7KO_2$) - are some of the most commonly used preservatives in food, owing to their favourability to low pH or acidic foods (El-Ziney, 2009; Akbari-adergani et al., 2013; Amirpour et al., 2015). In particular, sodium benzoate and potassium sorbate have been noted to be efficient in inhibiting the growth of moulds, yeast and a huge variety of bacteria (Mroueh et al., 2008). When used as preservatives, sodium benzoate and potassium sorbate are generally within the ranges of 0.05–0.1% and 0.02–0.3%, respectively (Mroueh et al., 2008). The ideal everyday intakes of sodium benzoate and potassium sorbate have been set respectively at 0–5 and 0–25 mg kg^{-1} of body weight (EFSA, 2017). Instigated by the effectiveness and affordable cost of these preservatives, some food manufacturers, however, have been observed to exceed the acceptable limit.

Value-adding ingredients (such as fruit or vegetable) may be incorporated into yoghurt to enhance its nutritional, functional or sensory value. For example, Ihemeje et al. (2015) investigated the nutritional profiles of yoghurt samples flavoured with carrot and pineapple, and yoghurt samples spiced with ginger and pepper. The authors found a significant nutritional enhancement in the flavoured and spiced yoghurts as compared to plain yoghurt. Similarly, Teshome et al. (2017) observed a significant improvement in the sensorial and physicochemical properties of mango and papaya flavoured yoghurts. Currently, only exotic fruits such as vanilla, strawberry, peach, raspberry, and banana types of flavoured yoghurts are commercially available. Soursop (*Annona muricata*) is a juicy, acidic, and aromatic tropical fruit with many therapeutic and nutritive properties (Senadeera et al., 2018). It is highly rich in fructose and vitamins C, B_1, and B_2 (Omoifo, 2004; Alias, 2009; Badrie & Schauss, 2010). Soursop is an underutilized tropical fruit in West Africa most especially Nigeria. However, since some health-conscious consumers are now reducing the rate of consumption of sugar-containing foods due to certain health concerns such as obesity, diabetes etc. associated with sugar consumption, replacing some percentage of sugar with an aromatic nature fruit such as soursop puree with no or acceptable use of preservatives in yoghurt production could lead to the development of functional yoghurt. Few valuable studies have been conducted on the potentials of soursop in enhancing the nutritional, organoleptic or functional quality of yoghurt. For instance, Senadeera et al. (2018) use pulp of three different varieties of soursop to produce yoghurt under different conditions. It was reported that the addition of soursop pulp to yoghurt increases the antioxidant acitivity and sensory profile of the yoghurt. Sputrayadi et al. (2021) noted that, in addition to enhanced organoleptic properties, the vitamin C and lactic acid levels of sweetcorn milk yoghurt significantly increase with an increasing ratio of soursop fruit extract. Sanusi et al. (2022) studied the kinetic acidification profile of yoghurt produced from soursop puree. Notwithstanding and to the best of our knowledge, the literature is still sparse on the interactive impacts of pasteurization temperature, chemical preservatives (sodium benzoate and potassium sorbate), soursop puree/sugar ratio, and storage duration on the qualities of set-type yoghurt. This study intend to produce set-type yoghurt that would provide valauable information on the impacts pasteurization temperature, chemical preservatives, storage duration and substitution of soursop puree with sugar on the syneresis, water holding capacity, proximate compostion, Vitamin C, total phenolic content, 2, 2 - Diphenyl – 1-picrylhydrazyl (DPPH), lactic acid bacteria emumeration, coliform emumeration, yeast and mould emumeration. This information would be useful is the selection of appropriate processing parameters and conditions that could aid in the production of nutritional and shelf-stable set-type yoghurt. Therefore, in this study aimed to evaluate the impacts of pasteurization temperature, use of chemical preservatives, soursop puree to sugar ratio, and storage duration on the physicochemical, antioxidant and microbial properties of set-type yoghurt.

2. Materials And Methods

2.1 Materials

The soursop fruits (*Annona muricata* L) used for this study were purchased from Harmony Farms (Ilorin, Nigeria), skimmed milk powder was purchased from Dano Milk, Arla Foods (Lagos, Nigeria), sugar was purchased from Dangote sugar (Ilorin, Nigeria) and freeze-dried yoghurt Lactic Acid Bacteria (LAB) starter culture containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilius* was used. The preservatives (sodium benzoate and potassium sorbate) were bought from Azchem resources, Lagos State, Nigeria.

2.2 Soursop puree

Mature, firm soursop (*Annona muricata* L) fruit was washed, peeled, and de-seeded. The fruit pulp was produced by blending the peeled and de-seeded soursop fruit with an electric blender (Model: SCB-505, China) until a smooth soursop puree was obtained. The sour puree was packaged and refrigerated before use.

2.3 Experimental design and production of set-type yoghurt

Taguchi orthogonal array $L_9^{(3**4)}$ was used to design the experiment using Minitab 16 statistical software. Table 1 shows the Taguchi orthogonal experimental design process parameters and levels for the production of set-type yoghurt. This was used to evaluate the impacts of pasteurization temperature, the concentration of soursop/sugar ratio, preservatives and storage duration on the physicochemical and microbial properties of the set-type yoghurt. A total of nine yoghurt treatments were generated from the experimental design.

Table 1
Taguchi experimental design process parameters and levels for production of set-type yoghurt

Process Parameters	Code	Unit	Level 1	Level 2	Level 3
Pasteurization temperature (°C)	A	°C	80	90	100
Soursop puree to sugar ratio (%)	B	%	0	25	50
Preservatives	C	type	No preservative	Sodium benzoate	Potassium sorbate
Storage duration	D	days	7	14	28

Set-type yoghurt used in this study was produced based on the modified procedure demonstrated by Sanusi et al. (2022). The ingredients used for the formulation of the set-type yoghurt were; skimmed milk, soursop puree, sugar, culture, preservatives (sodium benzoate and potassium sorbate) and water, respectively. The ingredients for control (plain) set-type yoghurt were correctly weighed using an electric weighing scale (Model: Cammry, China) as 1500 g of skimmed milk, 800 g of sugar, 10,000 litres of water and 5 g of freeze-dried starter culture, respectively. The concentrations of sugar to soursop puree ratio were varied at 0% (100% sugar and no soursop), 25% and 50%. The mixture of sugar and soursop puree, and the skimmed milk were dissolved in water at pasteurization temperature 80°C, 90°C and 100°C, and then thoroughly homogenized using a hand mixer (Model: OEM, China) to obtain a uniform product. The homogenized mixture was allowed to cool to 45^{o}C which is an ideal temperature for the starter culture, before introducing the inoculum. The inoculated samples were filled into polypropylene bottles and then transferred into a patented apparatus for conditioning dough and inoculated milk (Patent: NG/P/2020/199) as shown in Fig. 1. The inoculated products were incubated for 7 h at 40 ± 2^{o}C and the yoghurt incubation was terminated when the pH reached 4.6. Sodium benzoate and potassium sorbate was added at 0.05% and 0.02% seperately to the total mass of the yoghurt formed based on the experimental design in Table 1. The yoghurt samples were refrigerated at 4°C for 7, 14 and 28 days before further analysis.

2.4 Physicochemical Properties

2.4.1 Syneresis

The syneresis (%) of yoghurt samples was determined by centrifugation procedure using a modified version as described by Joung et al. (2016). After the desired storage duration, the syneresis was determined by centrifuging 20 g of yoghurt in 50 ml glass tubes at 3500 rpm for 15 min at 20°C. The syneresis was estimated using Eq. 1.

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

2.4.2 Water holding capacity

The water holding capacity was evaluated by using the modified method of Guzman-Gonzalez et al. (1999). A sample of about 20 g of yoghurt (*Y*) was centrifuged at 1250 x g for 10 min at 4^{0}C. The whey expelled (*W*) was removed and weighed. The water holding capacity (WHC, g.kg^{-1}) was estimated using Eq. 2:

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

2.4.3 Proximate composition

The moisture, ash, crude protein, fat, and fibre contents of the yoghurt samples were determined using AOAC official methods (2010). The total carbohydrate content was estimated by subtracting the total percentages of moisture, ash, crude protein, fat, and fibre from 100.

2.4.4 Vitamin C determination

The titrimetric method as described by Joy and Saumya (2013) was used to determine the Vitamin C content of the samples using Eq. 3.

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

2.5 Antioxidant Properties

2.5.1 Determination of Total Phenolic Content (TPC)

Measurement of TPC was carried out using the slightly modified procedure described by Zhou et al. (2004). A 400 µL of 2% 2 N Folin-Ciocalteu's phenol reagent and 800 µL of 10% Na_2CO_3 were mixed with a 200 µL appropriately diluted yoghurt sample. The mixture was then incubated for 3 minutes. Thereafter, the mixture was covered with an aluminium foil and held for 1 hour at room temperature (25 ± 2^oC) in the absence of light. The mixture was vortexed and its absorbance was measured at 750 nm using a microplate reader. A stanadard curve was then produced using gallic acid. Hence, measurements were expressed in milligrams of gallic acid equivalent per liter (mg GAE/l).

2.5.2 Determination of DPPH (2, 2 - Diphenyl – 1- picrylhydrazyl) radical-scavenging activities

The DPPH was evaluated using the approach of Zhang et al. (2016). The yoghurt supernatant and DPPH reagent of 0.01 mM were mixed in a plate of 96-well and allowed to react in the absence of light at room temperature (25 ± 2^oC) for 30 min. The control was established with DPPH reagent added in ethanol. Eq. 4 was used to determine the DPPH scavenging activity.

$$DPPH\ Scavenging\ (\%) = \left[1 - \left(\frac{Abs\_sample}{Abs\_control}\right)\right] \times 100\% \qquad (4)$$

2.6 Microbial Analysis

2.6.1 Lactic Acid Bacteria (LAB) Enumeration

Lactic acid bacteria in the samples were enumerated based on Miles-Misra method as described by Baron et al. (2006). The required dilution was prepared using sterile normal saline in which M17 and MRS agar media were used in culturing *S. thermophilus* and *L. bulgaricus*, and incubation took place at 42 and 48°C respectively, with 10% carbon dioxide for 48 hours (Baron et al., 2006).

2.6.2 Coliform enumeration

Hervert et al. (2017) approach was used to determine the coliform enumeration by mixing 1 ml of the yoghurt dilution 10^{-1} with a sterile MacConkey Agar in a Petri dish, thereafter the medium was allowed to set. Poured onto it was another layer of MacConkey agar and then incubation took place at 37°C for 24–48 hours.

2.6.3 Yeast and mould enumeration

The enumeration of yeast and mould was done by using Pasteur pipette and sterile potato dextrose agar as the culture medium and 1 ml of 10^{-1} diluted yoghurt sample (1 ml) was dispersed on the surface. The plates were incubated at 25°C for 5–7 days (Taniwaki et al., 2001).

2.7 Data Analysis

Data recorded for the nine treatments were analysed statistically using Duncan's new multiple range test of Analysis of Variance (ANOVA) (SPSS 22, IBM) at a 95% confidence level.

3. Results And Discussion

3.1 Effect of treatment conditions on syneresis and water holding capacity (WHC) of yoghurt

Syneresis and water holding capacity (WHC) are vital attributes of yoghurt that could limit its acceptability and shelf stability (Sidira et al., 2017). While syneresis is an undesirable attribute that describes the separation of liquid from the yoghurt curd, WHC measures the ability of the milk proteins to hold the water content of the yoghurt when force is applied. As the WHC of a yoghurt sample increases, the syneresis effect ideally reduces, although this correlation may be affected by the low pH value associated with yoghurt making (Wu et al., 2000). In this study, we noted a strong correlation (R^2 = 0.7, Adjusted R^2 = 0.65) between the WHC and the syneresis effect (Fig. 2a). The impact of pasteurization temperature, soursop to sugar ratio, preservation and storage duration on syneresis and WHC of yoghurt is shown in Fig. 2b. The syneresis from the yoghurt samples ranged from 12.00 ± 2.83 to 30.00 ± 2.83% while the WHC ranged from 44.70 ± 0.14 to 49.95 ± 0.21%. While treatment F had the lowest syneresis value (12.00 ± 2.83%) at 80°C pasteurization

temperature, 50% soursop puree, 28 days storage duration and when potassium sorbate was used as a preservative, treatment I had the highest WHC (49.95 ± 0.21%) at 100°C pasteurization temperature, 50% soursop puree, 7 days storage duration and when sodium benzoate was used as a preservative. However, there was no significant difference between the WHC of treatments I and F. On the other hand, the highest syneresis value (30.00 ± 2.83%) and lowest WHC was observed in treatment A at 80°C pasteurization temperature, 0% soursop puree, 7 days storage duration and when no preservative was used. Treatments E, F and I show no significant differences in their syneresis value and the low syneresis observed in these treatments could be attributed to the inclusion of soursop puree. The significant differences ($p < 0.05$) between treatments A and I may be attributed to the inclusion of soursop puree in treatment I contrary to treatment A. Our findings indicate that soursop puree incorporation could reduce syneresis and enhance the apparent viscosity of yoghurt. Previously, Mohammadi-Gouraji et al. (2018) have also noted that syneresis was higher in the control sample of yoghurt and lower in yoghurt enriched with phycocyanin. Likewise, Zhang et al. (2018) also observed that yoghurt supplemented with moringa extract had lower syneresis values and higher water holding capacity. Therefore, it could be hypothesized that increasing the total solid content of yoghurt increases the stability of the gel matrix (i.e., yoghurt curd), thereby leading to an increased water-holding capacity and reduced syneresis.

3.2 Effect of treatment conditions on proximate composition of yoghurt

The results of the proximate analysis of the nine yoghurt treatments are presented in Table 2. The moisture, ash, crude fibre, fat, protein and carbohydrate were ranges from 77.40–81.09%, 0.59–0.69%, 0.10–0.12%, 1.40–3.81%, 3.47–4.02% and 26.77–29.26%, respectively. Below 100 ºC pasteurization temperature, the addition of soursop increased the moisture content of the yoghurt samples. However, at 100ºC pasteurization temperature, there was a reduction in the moisture content, despite the addition of soursop. This may be due to the evaporation effect at 100ºC. The ash content also increased with the addition of soursop or preservatives. The increase might have come from the mineral contents of the preservatives or soursop puree. Crude fibre levels were not influenced by the treatment conditions. Moreover, the crude fibre levels in the yoghurt treatments were generally low and were similar to the findings of Amna et al. (2008). Adding soursop puree/sugar ratio up to 25% but not beyond increased the crude fat levels of the yoghurt samples, even though the crude fat levels were generally low, owing to the limited fat levels in soursop puree and skimmed milk (Amna et al., 2008). Increasing the soursop puree/sugar ratio up to 50% raised the crude protein and carbohydrate levels slightly while no effect of pasteurization temperature on protein denaturation was observed. Soursop protein level is given as 2.91% (Enweaeni et al., 2004). The effect of storage duration on the proximate composition was imperceptible. This may be due to the effectiveness of the refrigerated storage condition.

3.3 Effect of treatment conditions on vitamin C content of yoghurt

The results of vitamin C contents of the yoghurt treatments are presented in Fig. 3a. The vitamin C values range from 40.05 ± 0.35 mg/100g to 58.50 ± 0.07 mg/100g. The addition of soursop puree increased the vitamin C content. This could be as a result of the high vitamin C content of soursop fruit. Soursop has a vitamin C content of 29.6 mg/100g (Morton, 1987). Vitamin C levels were noted to reduce significantly in most cases at pasteurization temperature above 80ºC. This is expected as vitamin C is a heat-labile micronutrient - at higher temperatures, it undergoes a faster chemical reaction and degrades. Uckiah et al. (2009) also noted that a temperature treatment of 90 ºC resulted in decrease in vitamin C level in processed pineapple.

Table 2
Impact of pasteurization temperature, soursop puree to sugar (SP/SG) ratio, preservatives and storage duration on proximate composition of set-type yoghurt

Treatment	Pasteurization Temp. (°C)	SP/SG (%)	Preservative	Storage Duration (days)	Moisture Content (%)	Ash (%)	Crude Fiber (%)	Crude Fat (%)	Crude Protein (%)	CHO (%)
A	80	0	No preservative	7	77.40 ± 0.01^{c}	0.59 ± 0.01^{e}	0.10 ± 0.00^{a}	1.40 ± 0.01^{c}	3.75 ± 0.01^{e}	27.08 ± 0.05^{c}
B	80	25	sodium benzoate	14	81.07 ± 0.04^{a}	0.60 ± 0.01^{e}	0.11 ± 0.01^{a}	3.71 ± 0.29^{ab}	3.96 ± 0.01^{b}	27.26 ± 0.06^{c}
C	80	50	Potassium sorbate	28	81.09 ± 0.01^{a}	0.61 ± 0.01^{d}	0.11 ± 0.01^{a}	3.66 ± 0.35^{ab}	4.02 ± 0.01^{a}	28.71 ± 0.03^{ab}
D	90	0	sodium benzoate	28	79.41 ± 0.01^{b}	0.63 ± 0.01^{c}	0.11 ± 0.01^{a}	1.40 ± 0.01^{c}	3.88 ± 0.01^{c}	26.77 ± 0.04^{c}
E	90	25	Potassium sorbate	7	79.38 ± 0.01^{b}	0.67 ± 0.01^{b}	0.11 ± 0.01^{a}	3.48 ± 0.06^{b}	3.81 ± 0.01^{d}	27.21 ± 0.07^{c}
F	90	50	No preservative	14	81.07 ± 0.04^{a}	0.69 ± 0.01^{a}	0.12 ± 0.01^{a}	3.67 ± 0.01^{ab}	4.02 ± 0.01^{a}	28.67 ± 0.01^{b}
G	100	0	Potassium sorbate	14	79.28 ± 0.04^{b}	0.59 ± 0.01^{e}	0.10 ± 0.01^{a}	1.40 ± 0.01^{c}	3.47 ± 0.01^{f}	27.34 ± 0.69^{c}
H	100	25	No preservative	28	79.20 ± 0.01^{b}	0.63 ± 0.01^{c}	0.11 ± 0.01^{a}	3.81 ± 0.00^{ab}	3.99 ± 0.01^{a}	28.85 ± 0.03^{ab}
I	100	50	sodium benzoate	7	78.87 ± 0.71^{b}	0.69 ± 0.01^{a}	0.11 ± 0.01^{a}	3.40 ± 0.01^{a}	4.02 ± 0.01^{a}	29.26 ± 0.05^{a}

Values are mean ± standard deviation of two replicates. Means with different superscript letters along the same column are significantly different at $p \leq 0.05$. Where CHO is carbohydrate content.

The use of preservatives was only effective when the yoghurt samples were pasteurized at 80 ºC - but not beyond - as the levels of vitamin C were slightly higher in treatments containing preservatives as compared to the no preservatives treatments, although it is indiscernible which preservative type was more effective. Storage duration showed less or no noticeable effect on the vitamin C levels of the treatments. Again, this may be due to the effective refrigerated storage conditions.

3.4 Effects of treatment conditions on total phenolic content of yoghurt

Anti-ageing, anti-inflammatory, anti-oxidant, and anti-proliferative agents are some of the merits that have been observed with total phenolic content (TPC). TPC has also been reported to prevent the development of long-term diabetes complications. The results of TPC levels as observed in the yoghurt treatments are presented in Fig. 3b. Generally, the TPC levels of treatments containing soursop were higher as compared to those with no soursop. The observed values were in the range of 1.07 ± 0.01 mg GAE/l to 1.25 ± 0.00 mg GAE/l. The highest value (1.25 ± 0.00 mg GAE/l) was observed in treatment I at 100^{o}C pasteurization temperature, 50% soursop puree, 7 days storage duration and when sodium benzoate was used as a preservative. The least value (1.07 ± 0.01 mg GAE/l) was seen in treatment A at 80°C pasteurization temperature, 0% soursop puree, 7 days storage duration and when no preservative was used. The total phenolic content of yoghurt with soursop was higher than that of the control (0% soursop) treatments. This is probably because of the phenolic compounds present in soursop fruit (Senadeera et al., 2018).

3.5 Effect of treatment conditions on DPPH radical scavenging activity of yoghurt

DPPH radical scavenging activity is used as a pointer to the antioxidant capacities of bioactive compounds in a given sample (Najgebauer-Lejko et al., 2011). Antioxidants are compounds that can scavenge and inactivate free radicals that cause damages to cells and tissues, leading to degenerative diseases (Rafieian-Kopaei et al., 2013). The results of DPPH radical scavenging activity levels as observed in the yoghurt treatments are presented in Fig. 3c. The DPPH values ranged from 29.68 ± 0.01 to 34.40 ± 0.19% with the least value observed in treatment D at 90^{0}C pasteurization temperature, 0% soursop puree, 28 days storage duration and when sodium metabisulphite was used for preservation, and the highest value recorded for treatment I at

100^0C pasteurization temperature, 50% soursop puree to sugar ratio, 7 days storage duration and when sodium metabisulphite was used for preservation.

DPPH radical scavenging activities were significantly higher ($p < 0.05$) in yoghurt treatments with soursop puree as compared to the yoghurt treatments without soursop. This can be attributed to the amounts of vitamin C and total phenolic content present in the soursop. This suggests that the treatments with soursop puree will have higher antioxidant capacities and impact better health-promoting properties than the yoghurt treatments without soursop puree. Similar findings were reported by Nguyen and Hwang (2016) who concluded that the antioxidant capacities of yoghurts flavoured with Aronia (*Aronia melanocarpa*) were higher than that of plain yoghurt. Comparably, the addition of sour cherry pulp and strawberry pulp significantly have been noted to increase the antioxidant capacity of yoghurt (Sengul et al., 2012; Oliveira et al., 2015).

3.6 Effects of treatment conditions on microbial properties of yoghurt

The results of microbial analysis of the yoghurt treatments are presented in Fig. 4. As expected, the growth of lactic acid bacteria (LAB) dominated the microbial population. Some considerable counts of moulds and yeasts were also detected.

3.6.1 Total coliform counts

There were no coliform counts in all the yoghurt treatments produced. This observation was similar to the findings of Younus et al. (2002). Contrarily, El Bakir and El Zubeir (2009) reported a higher mean coliform of 3.95 ± 4.35 log CFU/ml. It is important to state that local and CODEX standards permit no coliforms at all. The reason for no coliform count in this study could be due to the hygienic conditions used in production to prevent cross-contamination. Therefore, regardless of the low coliform counts reported in those studies, they were not indicative of products of acceptable microbial quality (Codex standard 243–2003).

3.6.2 Lactic acid bacteria counts

The growth of lactic acid bacteria was in the range of 2.9×10^5CFU/ml to 2.16×10^6 CFU/ml. Treatment I had the highest value (2.16×10^6 CFU/ml) at 100^oC pasteurization temperature, 50% soursop puree to sugar ratio, 7 days storage duration and when sodium benzoate was added as a preservative. Treatment C had the lowest LAB count at 80^oC pasteurization temperature, 50% soursop puree to sugar ratio, 28 days storage duration and when potassium sorbate was used as a preservative. It was observed that the storage duration of the yoghurt treatments had the most influence on the lactic acid bacteria count. Also, treatment E with a storage duration of 7 days had the second-highest lactic acid bacteria count and treatment D second lowest count was stored for 28 days. It could be deduced from this study that there was an inverse relationship between that the storage duration of the treatments and lactic acid bacteria count. As the storage duration increased, the lactic acid bacteria count reduced, implying that aged yoghurt contains lesser active lactic acid bacteria than the freshly produced treatment. Similar findings were reported by Rao et al. (1987) in which lactic acid bacterial count of labneh (concentrated yoghurt) decreased from 10^7 CFU/g to 10^3 CFU/g after 6 months of storage.

3.6.3 Total mould counts

The range of total mould counts was between 3.05×10^5 CFU/ml to 6.5×10^4CFU/ml. The highest value (3.05×10^5 CFU/ml) was observed in treatment G at 100^oC pasteurization temperature, 0% soursop puree, 14 days storage duration and when potassium sorbate was used as a preservative. Treatment F had the lowest value (6.5×10^4 CFU/ml) at 90^oC pasteurization temperature, 50% soursop puree, 14 days storage duration and with no added preservative. It was discovered that the preservative had an effect on the mould count in each yoghurt treatment, particularly treatments incorporated with potassium sorbate had the highest mould counts. The treatments with the lower mould counts were produced with sodium benzoate preservatives. This shows that sodium benzoate is more active in inhibiting the mould counts in the yoghurt treatments.

3.6.4 Total yeast counts

The total yeast count value ranges from 1.0×10^6 CFU/ml to 1.2×10^5 CFU/ml. It was observed that treatment E had the highest yeast count (1.0×10^6 CFU/ml) at 90^oC pasteurization temperature, 25% soursop puree, 7 days storage duration and when potassium sorbate was included as a preservative. The least yeast count (1.2×10^5 CFU/ml) was observed in treatment D at 90^oC pasteurization temperature, 0% soursop puree, 28 days storage duration and when sodium benzoate was added as a preservative. The preservative used influences the yeast counts of the yoghurts. Treatment E was produced with potassium sorbate which shows its inactiveness in inhibiting the yeast growth while treatment D was produced with sodium benzoate which shows its activeness in inhibiting the yeast growth. A similar trend was observed for other yoghurt treatments as well.

4. Conclusions

In this study, we investigated the effects of pasteurization temperature, use of chemical preservatives, soursop puree substitution with sugar at different ratio, and storage duration on the physicochemical, nutritional, anti-oxidative and microbial properties of set-type yoghurt. It was found out that, by increasing the soursop puree/sugar ratio, the water holding capacity of the yoghurt increases while the syneresis decreases. A strong correlation was also observed between the water holding capacity and the syneresis. The incorporation of 50% soursop puree to sugar ratio in the yoghurt treatments gives rise to higher crude fat, carbohydrate, protein, ash and vitamin C contents and increased levels of total phenolic content and DPPH activity. The moisture content of the yoghurt treatments reduces with an increase in soursop puree to sugar ratio. Pasteurization temperature above 80ºC significantly degraded vitamin C content. No coliform was detected with or without preservatives. The use of sodium benzoate as a preservative at an acceptable

permissible limit gives a better result in inhibiting the growth of mould and yeast than potassium sorbate in the yoghurt treatments. The lactic acid bacteria count decreases with extending storage duration. In our future studies, optimization of the impacts of the treatment conditions on the quality attributes of set-type yoghurt would be considered. The outcome of our study will be of great value to food product and process development, and quality control.

Declarations

Authors contribution: Dr. Sanusi designed the experiment, wrote the main manuscript and the reviewed manuscript. Dr. Sunmonu supervised and reviewed the manuscript. Dr. Raji and Mr. Alaka prepared the figures and reviewed the manuscript. Mr. Abdulazeez and Miss Victoria conducted the experiment and reviewed the manuscript.

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Conflict of Interest Statement: There is no conflict of interest among the authors.

Data Availability Statement: Data available upon request.

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Figures

Figure 1

Apparatus for Conditioning Dough and Inoculated Milk (Sanusi & Sumonu, 2020)

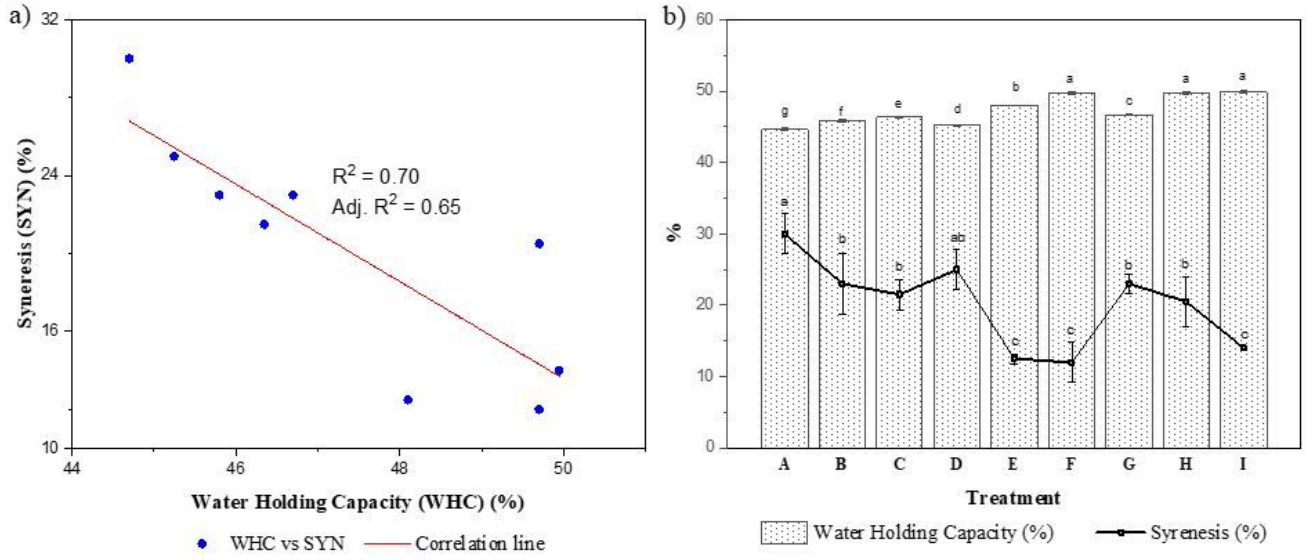

Figure 2

(a) Correlation between observed syneresis and water holding capacity (WHC) of yoghurt. (b) Effects of pasteurization temperature (PT), soursop puree/sugar ratio (SP/SG), preservatives (PV) and storage duration (SD) on syneresis and WHC of yoghurt samples. Letters A-I represent different treatment conditions including PT (°C), SP/SG (%), PV (-) and SD (days): A–80, 0, No preservative (NP), 7; B–80, 25, sodium benzoate (SB), 14; C–80, 50, potassium sorbate (PS), 28; D–90, 0, SM, 28; E–90, 25, PS, 7; F–90, 50, NP, 14; G–100, 0, PS, 14; H–100, 25, NP, 28; I–100, 50, SM, 7. Results are average (plus/minus standard deviation) of duplicate experiments. Means with different superscript letters are significantly different ($P < 0.05$).

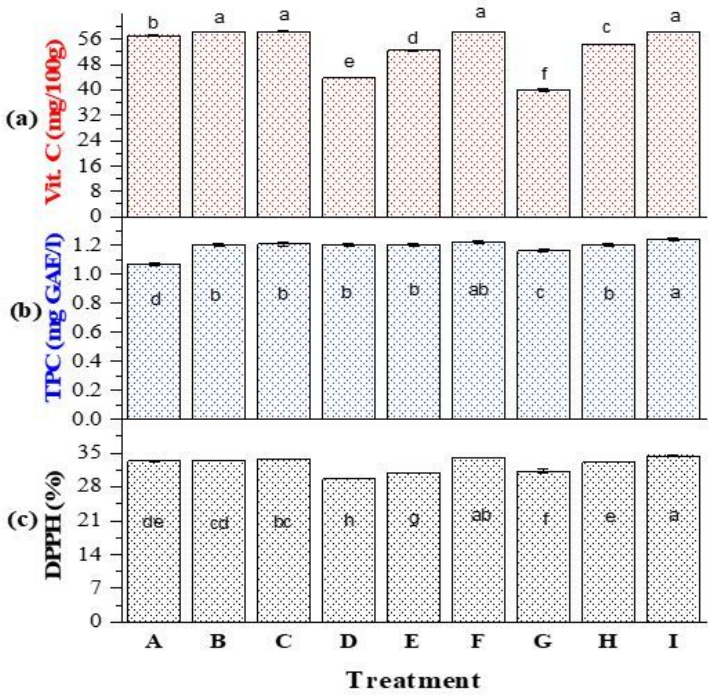

Figure 3

Effects of pasteurization temperature (PT), soursop puree/sugar ratio (SP/SG), 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. Letters A-I represent different treatment conditions including PT (°C), SP/SG (%), PV (-) and SD (days): A–80, 0, No preservative (NP), 7; B–80, 25, sodium benzoate (SB), 14; C–80, 50, potassium sorbate (PS), 28; D–90, 0, SM, 28; E–90, 25, PS, 7; F–90, 50, NP, 14; G–100, 0, PS, 14; H–100, 25, NP, 28; I–100, 50, SM, 7. Results are average (plus/minus standard deviation) of duplicate experiments. Means with different superscript letters are significantly different ($P < 0.05$).

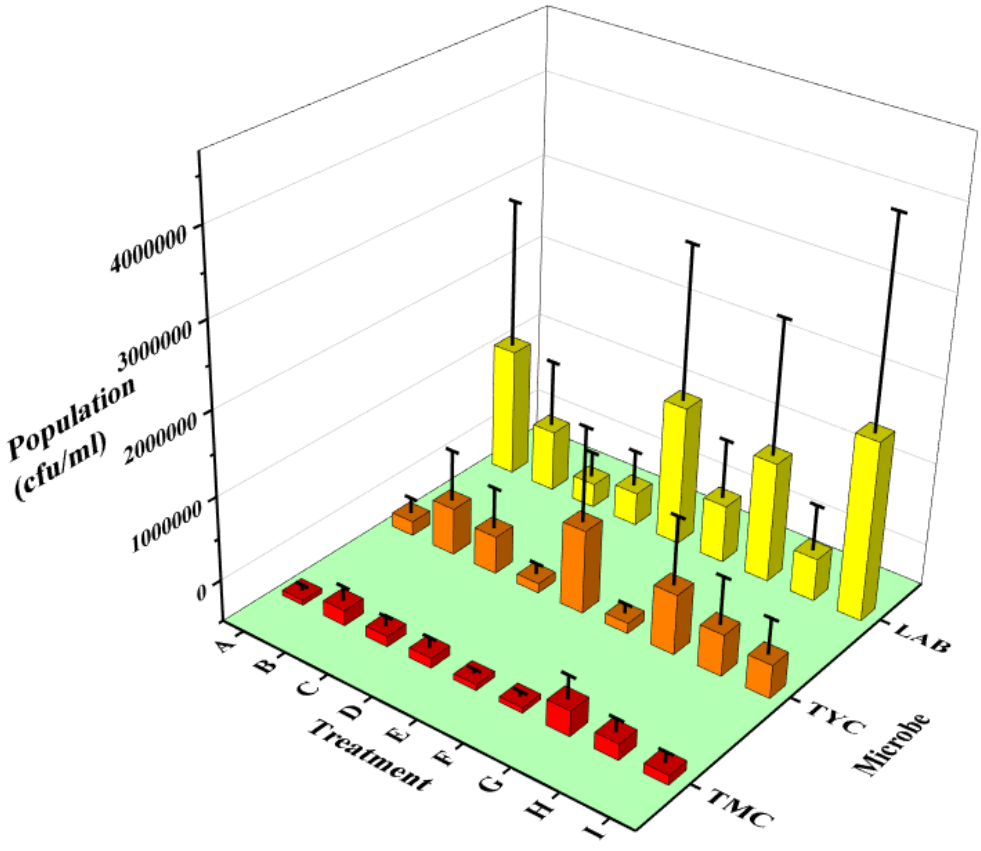

Figure 4

Effects of pasteurization temperature (PT), soursop puree/sugar ratio (SP/SG), preservatives (PV) and storage duration (SD) on Lactic Acid Bacteria (LAB), total mould (TMC) and total yeast (TYC) counts of yoghurt samples. Letters A-I represent different treatment conditions including PT (ºC), SP/SG (%), PV (-) and SD (days): A–80, 0, No preservative (NP), 7; B–80, 25, sodium benzoate (SB), 14; C–80, 50, potassium sorbate (PS), 28; D–90, 0, SM, 28; E–90, 25, PS, 7; F–90, 50, NP, 14; G–100, 0, PS, 14; H–100, 25, NP, 28; I–100, 50, SM, 7. Results are average (plus/minus standard deviation) of duplicate experiments. Means with different superscript letters are significantly different ($P < 0.05$).