Biochemical and Sensory Characteristics of Dairy-Tigernut Yoghurt using Ginger as Bio-Preservative

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KEYWORDS

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ABSTRACT

Tiger-nut milk is a good substitute for cow milk in yoghurt production because of its health benefit, accessibility and availability especially for people living in low-income countries, people living with lactose intolerance and vegetarian. Ginger extract (4%) was added as a bio preservative to improve the keeping quality of products obtained. Effect of ginger extract was studied on both tiger-nut yoghurt, cowmilk yoghurt and the blends. Microorganisms were identified during storage, total sugar and titratable acidity were evaluated and organoleptic properties were assessed. Microbial count was lower in samples with ginger extract and pathogenic microorganisms were not detected during storage. Microorganisms isolated were Lactobacillus delbrueckii, Streptococcus thermophilus, Lactobacillus plantarum, Pediococcus acidilactic, Saccharomyces cerevisiae, Leuconostoc mesenteroides, Bacillus subtilis, Staphylococcus epidermidis, Aspergillus niger and Micrococcus roseus. Tiger-nut generally increased the total sugar and pH of the samples and reduced the titratable acidity. These findings suggested that addition of ginger can increase the keeping quality of dairy, tiger-nut yoghurt and their blends without altering the organoleptic properties thereby providing healthy diet and reducing nutritional related disease.

INTRODUCTION

Yoghurt is usually obtained from cow milk; though ewe, goat and buffalo also supply appreciable quantity of milk. Animal milk are expensive and not accessible to everyone so, there is need to explore alternative source of milk to meet the demand of the teaming population in order to improve their micronutrient intake, nutritional and health status. Lactose intolerance, religious believe and vegetarianism are other factors for exploring milk and milk product from plant source (Malomo and Abiose, 2019). Yoghurt is the most popular probiotic containing food because of its attractive appearance, texture, desirable taste and consumer's satisfaction (Ogundipe *et al.*, 2021). The probiotic microorganisms involved in fermentation of yoghurt have been reported to improve the health of the consumer by having positive effect on metabolic rate, absorption of nutrients, immune function, and maintaining intestinal microbial balance (Ogundipe *et al*., 2021). Milk and yoghurt analogue has been produced from soy milk, mung beans, coconut and tiger-nut (Onyimba, 2022).

Tiger-nut (*Cyperus esculentus*) is a tuber crop which is usually consumed raw or processed into products such as milk, meal and powder. It contains starch, oil rich in oleic acid, essential micronutrients (calcium, magnesium, iron and phosphorus), amino acids, vitamins E and C. It is used as additive in ice cream production, in composite flour for making biscuits, bread and other pastries and in production of beer and liquor (Bamishaye and Bamishaye, 2011; Jing *et al*., 2016; Ogo *et al*., 2018). Tiger-nut milk is popular in Spain and Nigeria where it is produced in household quantities, small scale and large scale. It is a light brown coloured non-alcoholic drink obtained from aqueous extract of tiger-nut tuber. It is consumed at home, during ceremonies, and sold in restaurants and supermarkets. The beverage is also gaining more attention around the world because of its nutritional benefit and organoleptic properties. Milk from tiger-nut is rich in starch, oleic acid, linoleic acid and arginine (Bamishaye and Bamishaye, 2011; Jonas *et al*., 2019). Milk obtained from tiger-nut into different milk products like pasteurized milk and condensed milk and it is suitable for people with cow milk allergies. The biological value of its protein content is high and it is higher than the recommended standard of FAO/WHO for adult (Ukwuru and Obodo, 2011).

ne of the challenges with tiger-nut milk is short shelf life because it cannot be heated above 72 °C during pasteurization to prevent jellification of starch (Jonas *et al*., 2019). Alertness on the use of safe additives by the consumers has increased the use of bio preservatives in foods. Spices such as ginger, garlic, *Xylopia aethiopica*, black pepper and cloves have been used to improve the organoleptic properties, microbiological characteristics and anti-oxidative activities of food. Ginger (*Zingiber officinale*) rhizome contain gingerol and shagoal which gives it desirable aroma and taste and also has therapeutic effect in the body (Malomo *et al*., 2020). Several authors have worked on the antibacterial, antifungal and antioxidant properties of ginger (Olaniran *et al*., 2018; Malomo and Abiose, 2020). Use of natural flavours could exert positive effect on the health of the consumer by serving as safe bio preserved functional food (Njoya *et al*., 2018). This research aims to study effects of ginger extract on the microbial population, types of microorganisms, safety and acceptability of dairy-tiger-nut yoghurt.

MATERIALS AND METHODS

Cow milk was obtained from Obafemi Awolowo University Teaching and Research farm, Ile – Ife, Nigeria. Tiger-nut and ginger were obtained from "Odoogbe" market, Ile – Ife. Media and chemical used for this research were of analytical grades.

Preparation of ginger

Ginger was washed, peeled, sliced and dried in hot air oven at 60 oC to 5 % moisture content and ground in a domestic blender. The powder obtained was sieved through 0.5 mm mesh and 100 g was soaked in 500 ml of distilled water. The mixture was stirred on magnetic stirrer and filtered with muslin cloth. The supernatant was dispensed into an airtight plastic and kept at 4 oC Modified method of Olaniran *et al*. (2019).

Production of tiger-nut milk

Method of Sanful (2009) was used for production of tiger-nut milk. Tiger-nut was cleaned, sorted, washed in portable water and soaked in water for 12 h. The water was drained, tiger-nut was rinsed in portable water and 450 g was ground in 1 L of water using a domestic blender. The mixture was double filtered through muslin cloth, residue was discarded and the milk was poured into a clean plastic container.

Production of dairy – tiger-nut yoghurt

Tiger-nut was mixed with cow milk at ratio 50:50, with or without addition of 4% ginger extract, 4% ginger extract was added to cow milk and 4% ginger extract was added to tiger-nut milk. The control samples are100% cow milk and 100% tiger-nut milk. These milk samples were pasteurized in water bath at 72 oC for 30 min, cooled to 43 oC, inoculated with 1.5% of starter culture and incubated in Gallen Kamp incubator for 10 h at 45 oC. Each sample was dispensed into sterile sampling bottle for analyses (modified method of Malomo and Abiose, 2020).

Table 1. Sample preparation

Code	Sample
DY	100 % cow milk yoghurt without ginger
TY	100% tiger-nut yoghurt without ginger
BY	50% tiger-nut, 50% cow milk yoghurt without ginger
DG	100% Cow milk yoghurt and + ginger
TG	100% tiger-nut yoghurt and + ginger
BG	50% tiger-nut, 50% cow milk yoghurt and + ginger

Microbiological analyses

Each sample (5 ml) was thoroughly mixed with peptone water (45 ml) in a stomacher and diluted appropriately. Appropriate dilution (1 ml) was pipetted in sterile plate and 20 ml of molten agar was poured. Total viable count (TVC) and lactic acid bacteria (LAB) were enumerated using Nutrient agar and MRS agar respectively. The petri dishes were incubated for 24 h at 35 °C for TVC and for 72 h at 35°C for LAB respectively. The colonies formed were counted and result was recorded in log colony forming unit per ml. Pure isolates were obtained by streaking colonies on appropriate agar and later streaked on agar slant in McCartney bottles and kept at 4 °C (Harrigan, 1998).

Appearance of colony on plates and morphological characteristics, Gram's staining and biochemical tests were used for the identification of isolate. Colony characteristics, shape of cell, size of cell, type of budding, assimilation of carbon and nitrate sources were used for identification of yeast isolate. Mould isolate was identified using the colour of spores, reproduction, type of hyphae and presence of special structures (Harrigan, 1998).

Determination of Total sugar content

Total sugar of yoghurt samples was determined using the anthrone reagent method described by Morris (1948). Glucose (0.01 g) was dissolved in distilled water (100 ml) to produce the stock solution for Standard sugar (0 - 1000µg). Yoghurt samples were diluted appropriately and double filtered through Watman 1 filter paper. The filtrate (1 ml) was pipetted into test tube containing 4 ml of anthrone reagent and boiled at 100 ^oC. The absorbance was read against reagent blank in spectrophotometer at 620 nm (Spectrumlab 752S, YM1206PHB2, China). Anthrone reagent (4 ml) was added to each sugar standard (1 ml) and the absorbance was obtained at 620 nm. The amount of sugar in each yoghurt samples was obtained from the standard glucose curve (Malomo *et al*., 2019).

Determination of Titratable acidity

Each sample (10 ml) was poured into conical flask and thoroughly mixed with distilled water (10 ml). Phenolphthalein indicator (3 drops) was added and was titrated against 0.1 N NaOH (AOAC, 2005).

Determination of pH

pH of the yoghurt samples was determined using pH meter (Philips model PHS-3C) which was calibrated using buffer 4 and 7. The electrode was inserted into each yoghurt sample and the readings displayed on screen was recorded (AOAC, 2005).

Sensory assessment

Freshly prepared yoghurt samples were coded and presented to fifteen panelists that were familiar with yoghurt. The samples were analyzed for colour, taste, flavour and overall acceptability using a 9 -point Hedonic (Montgomery, 2004).

Statistical analysis

The results were analyzed using SPSS (2010) for standard deviation and least significant difference. Principal Component Analysis, Agglomerative Hierarchical Clustering were analyzed using XLSTAT (2014).

RESULTS AND DISCUSSION

The frequency of the microorganisms is shown in Figure 1. *Lactobacillus delbrueckii* (25.3%), *Streptococcus thermophilus* (23.2%), *Lactobacillus plantarum* (12.4%), *Pediococcus acidilactic* (9.6%), *Saccharomyces cerevisiae* (12.6%), *Leuconostoc mesenteroides* (7.3%), *Bacillus subtilis* (4.9%), *Aspergillus niger* (1.2%), *Micrococcus roseus* (1.5 %) were isolated from the yoghurt samples during storage. *Lactobacillus delbrueckii, streptococcus thermophilus* and *Lactobacillus plantarum* were isolated from all samples from week 0 to week 4. *Leuconostoc mesenteroides* was isolated in all samples at week 0 and week 1. Malomo and Abiose (2020) also reported the viability of lactic acid bacteria in soy and dairy yoghurt. Lactic acid bacteria utilized the sugar present in yoghurt as carbon source and produce organic acids and volatile substances that improves the consistency and organoleptic properties of fermented food. They also reduce the pH and produce antimicrobial substances that inhibit pathogenic microorganisms (Malomo *et al*., 2020). *Pediococcus acidilactici* was isolated from TY, TG, BY and BG from week 0 – week 4 and *Saccharomyces cerevisiae* was isolated from week 1 to week 4. *Saccharomyces cerevisiae* was isolated from all yoghurt samples at the fourth week of storage. *Staphylococcus epidermidis* was isolated in TY at week 1 while *Micrococcus rubeus* and *Aspergillus niger* was isolated from TY at week 3 and week 4. All strains of *Pediococcus acidilactici* has ability to grow at 50°C but cannot withstand heating at 70 ^oC for 10 min (De Vos, 2009).

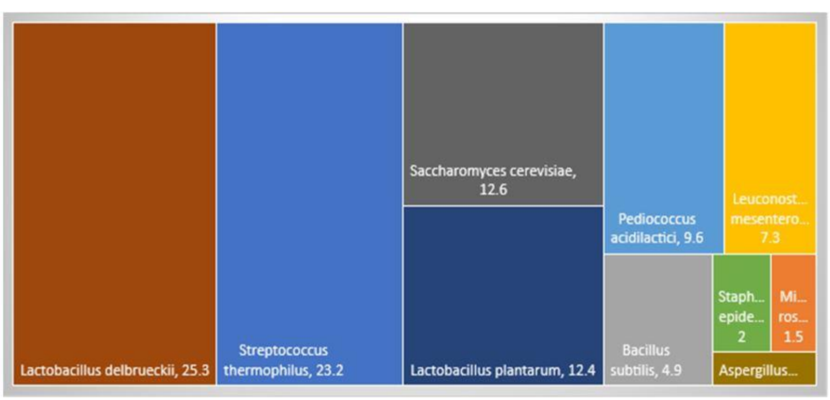

Figure 1. Percentage Occurrence of Microorganisms isolated from dairy - tiger-nut yoghurt during storage (%)

Total viable count of yoghurt

The TVC ranged between 3.85 to 9.38 log CFU/ml (Table 2). TY had the highest microbial count (8.20 log CFU/ml) at week 0 which was significantly different ($p < 0.05$) from the TVC of other samples. Addition of ginger significantly decreased the TVC of yoghurt samples produced from 100% tiger nut. At week 2, the TVC was highest in TY (9.38 log CFU/ml) followed by DY (8.410 log CFU/ml) and lowest in the BG (8.13 log CFU/ml). Addition of ginger significantly reduce ($p < 0.05$) the microbial count of all yoghurt samples from week 2 to week 4. The TVC of all yoghurt sample were within the acceptable range from week 1 to week 2. Samples DY, BY and DG containing cow milk still conform to the standard at week 4 (CODEX, 2011).

Table 2. Total viable count of dairy- tiger-nut yoghurt

			Storage Period		
Sample	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
DY	7.33 ± 0.04^{a}	8.40 ± 0.57^{a}	8.41 ± 0.01^{b}	5.72 ± 0.11^{ab}	7.35 ± 0.35^{a}
TY	8.20 ± 0.57^{b}	8.44 ± 0.03^{a}	9.38 ± 0.10^{a}	5.89 ± 0.08^{a}	5.08 ± 0.07^{c}
BY	7.34 ± 0.57^{a}	8.10 ± 0.57^{a}	8.15 ± 0.57^{cd}	5.41 ± 0.10^{c}	6.81 ± 0.09^{b}
DG	7.32 ± 0.03^{a}	8.29 ± 0.10^{a}	8.31 ± 0.14^{bcd}	5.54 ± 0.04^{cd}	7.18 ± 0.84^{ab}
TG	7.19 ± 0.13^{a}	8.17 ± 0.07^{a}	8.34 ± 0.03^{bc}	5.54 ± 0.28^{cd}	4.94 ± 0.28^{c}
BG	7.30 ± 0.28^{a}	7.95 ± 0.57^{a}	8.13 ± 0.21^{d}	5.34 ± 0.13^{d}	3.95 ± 0.21^{d}

DY: Dairy Yoghurt, TY: Tiger-nut Yoghurt, BY: 50% Dairy + 50% Tiger-nut, Yoghurt, DG: Dairy Yoghurt + 4% Ginger Extract, TG: Tiger-nut Yoghurt + 4% Ginger Extract, BG: 50% Dairy + 50% Tiger-nut Yoghurt + 4% Ginger Extract. Values are means of three replicates ± standard error. Means followed by different superscript in the same row are significantly different at $p<0.05$

The lactic acid bacteria count of the dairy – tiger-nut yoghurt

The count generally increased during storage from week 0 to week 2 and decreased from week 2 to week 3 (Table 3). TY (4.77 – 5.41 log CFU/ml) generally had significantly lower count ($p < 0.05$) than DY (4.95 – 6.41 log CFU/ml) from week 1 to week 2 while counts were higher in TY (5.34 – 5.15 log CFU/ml) than DY (5.18 – 5.08 log CFU/ml) from week 3 to week 4 but the difference was not significant ($p > 0.05$). Ginger extract reduced LAB count in the samples but the reduction was not significant ($p > 0.05$) in DY and DG from week 0 to week 4 and also in BY and BG from week 0 to week 4. This show that ginger extract is a suitable bio preservative for production of functional yoghurt. Malomo *et al*. (2020) also reported that ginger did not affect the viability of LAB in cheese.

Table 3. Lactic acid bacteria count of dairy – tiger-nut yoghurt

			Storage Period		
Sample	Week 0	Week 1	Week 2	Week 3	Week 4
DY	4.95 ± 0.19^{a}	6.28 ± 0.06^{a}	6.41 ± 0.10^{a}	5.18 ± 0.13^{b}	5.08 ± 0.05^{b}
TY	4.77 ± 0.13^{a}	5.00 ± 0.42^{b}	5.41 ± 0.01^{b}	5.34 ± 0.06^{ab}	5.15 ± 0.06^{ab}
BY	4.07 ± 0.10^{b}	6.80 ± 0.20^{a}	5.35 ± 0.05^{b}	5.32 ± 0.03^{ab}	5.11 ± 0.16^{b}
DG	4.77 ± 0.17^{a}	5.95 ± 0.07^{a}	4.54 ± 0.06^{c}	5.28 ± 0.07^{ab}	5.26 ± 0.04^{ab}
TG	4.85 ± 0.07^{a}	5.85 ± 0.04^{a}	5.27 ± 0.04^{b}	4.69 ± 0.06^{c}	4.51 ± 0.21^{c}
BG	4.90 ± 0.14^{a}	6.08 ± 0.08^{a}	5.30 ± 0.42^{b}	5.41 ± 0.18^{a}	5.33 ± 0.06^{a}

DY: Dairy Yoghurt, TY: Tiger-nut Yoghurt, BY: 50% Dairy + 50% Tiger-nut, Yoghurt, DG: Dairy Yoghurt + 4% Ginger Extract, TG: Tiger-nut Yoghurt + 4% Ginger Extract, BG: 50% Dairy + 50% Tiger-nut Yoghurt + 4% Ginger Extract. Values are means of three replicates ± standard error. Means followed by different superscript in the same row are significantly different at $p<0.05$

The total sugar content of dairy – tiger-nut yoghurt

The total sugar content of dairy – tiger-nut yoghurt ranged between 99.509 – 152.002 mg glucose ml from week 0 to week 4 (Table 4); it generally decreased during storage and was higher in TY than DY. The range of total sugar obtained was slightly higher than 2.0 – 11% reported by Ukwuru *et al* (2008). Addition of tiger-nut significantly increased the total sugar content of the dairy yoghurt during storage. Yoghurt samples containing ginger extract generally had higher content of total sugar. Total sugar was generally higher in yoghurt samples containing tiger nut (136.248 – 152.002 mg glucose/ml) than cow milk (99.509 - 151.391 mg glucse/ml). Higher sugar content of yoghurt samples containing tiger-nut is probably due to high carbohydrate content of the nut (Sani *et al*., 2019). Tiger-nut milk is an emulsion of oil droplets in an aqueous phase containing starch granules and other solid particles (Rosello-Soto *et al*., 2018).

Table 4. Total sugar content of cow milk – tiger-nut yoghurt during storage (mg glucose/ml)

	Storage Period				
Sample	Week 0	Week 1	Week 2	Week 3	Week 4
DY	151.391±0.141^{bc}	143.890±0.003^{d}	131.059±0.013^{f}	133.794±0.048^{c}	99.509±0.041^{f}
TY	152.002±0.003^{a}	148.096±0.035^{a}	146.133±0.014^{c}	142.207±0.017^{a}	136.248±0.068^{c}
BY	151.462±0.080^{b}	146.344±0.062^{c}	150.550±0.007^{a}	139.612±0.047^{b}	135.126±0.037^{d}
DG	151.195±0.071^{c}	139.613±0.028^{e}	134.565±0.354^{e}	133.864±0.020^{c}	117.878±0.028^{e}
TG	151.882±0.116^{a}	147.676±0.014^{b}	148.798±0.043^{b}	142.768±0.014^{a}	139.262±0.028^{b}
BG	151.601±0.030^{b}	146.414±0.018^{c}	144.100±0.014^{d}	139.683±0.004^{b}	140.244±0.020^{a}

DY: Dairy Yoghurt, TY: Tiger-nut Yoghurt, BY: 50% Dairy + 50% Tiger-nut, Yoghurt, DG: Dairy Yoghurt + 4% Ginger Extract, TG: Tiger-nut Yoghurt + 4% Ginger Extract, BG: 50% Dairy + 50% Tiger-nut Yoghurt + 4% Ginger Extract. Values are means of three replicates ± standard error. Means followed by different superscript in the same row are significantly different at $p<0.05$

Titratable acidity of dairy – tiger-nut yoghurt

The titratable acidity (TTA) of yoghurt samples is shown in Fig. 3. The TTA of the freshly prepared yoghurt samples was between 0.59 and 1.40 %. It was generally lower in yoghurt samples produced from tiger-nut and the blends (0.57 – 0.83%) than dairy yoghurt (1.40 – 2.03%) throughout storage period. Ginger extract generally facilitate acid production in yoghurt samples. Low TTA was also reported with increase in addition of tiger-nut milk by Ojuko *et al*. (2019).

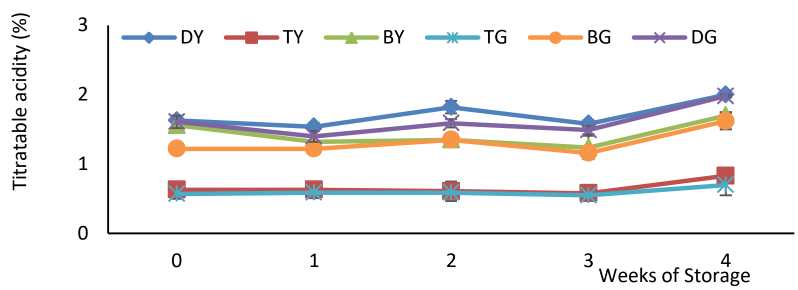

Figure 3. The titratable acidity of dairy – tiger-nut yoghurt during storage

DY: Dairy Yoghurt, TY: Tiger-nut Yoghurt, BY: 50% Dairy + 50% Tiger-nut, Yoghurt, DG: Dairy Yoghurt + 4% Ginger Extract, TG: Tiger-nut Yoghurt + 4% Ginger Extract, BG: 50% Dairy + 50% Tiger-nut Yoghurt + 4% Ginger Extract.

pH of dairy – tiger-nut yoghurt

The pH of yoghurt samples generally decreased with increase in storage time (Figure 4). It was highest in TY (5.76 – 6.00) which contain 100% tiger-nut throughout the period of storage while DY containing 100% cow milk (4.22 – 4.63) had the lowest. Addition of 4% ginger extract reduced the pH of both tiger-nut and cow milk yoghurt and their blends. The reduction in pH also led to reduction in both total viable count and lactic acid bacteria count of these samples. This also shows that ginger extract is a potential bio preservative in both plant and animal milk. The reduction in pH could be due to the activities of the microorganisms involved in the fermentation process. Since the samples were not pasteurized after production, the microorganisms continued utilizing the available carbon thereby converting it to simple sugars that were ultimately utilized as by microorganisms as source of nutrient. (Adepoju *et al*., 2012; Malomo et a., 2020). These microorganisms also produced organic acids such as lactic acid, acetic acid and butyric acid which also eliminates the growth of pathogenic microorganisms that can cause health challenges thereby establishing the safety of the food. Organic acid has been reported to have bacteriocidal and bacteriostatic effect on both spoilage and pathogenic microorganisms that might have adverse effect on the quality and safety of food (Adepoju *et al*., 2016).

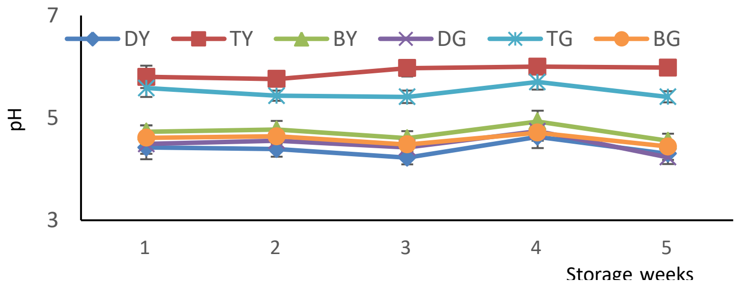

Figure 3. The titratable acidity of dairy – tiger-nut yoghurt during storage

DY: Dairy Yoghurt, TY: Tiger-nut Yoghurt, BY: 50% Dairy + 50% Tiger-nut, Yoghurt, DG: Dairy Yoghurt + 4% Ginger Extract, TG: Tiger-nut Yoghurt + 4% Ginger Extract, BG: 50% Dairy + 50% Tiger-nut Yoghurt + 4% Ginger Extract.

Sensory evaluation of dairy – tiger-nut yoghurt samples

Table 5 shows the results of the sensory properties of dairy-tiger-nut yoghurt. DY was scored highest for colour, taste, flavour and overall acceptability followed by DG. Scores obtained for DG and DY was not significant except for the taste which had significantly lower score ($p < 0.05$) in DG showing that 4% ginger extract had significant effect on the taste of dairy yoghurt. Addition of 4% ginger extract also significantly increased the texture of all yoghurt samples. The low score for yoghurt produced from tiger-nut may be due to the fact that the panelists are more familiar with dairy yoghurt than tiger-nut yoghurt. The yoghurt blends BY and BG had significantly higher score for taste, flavour and colour and overall acceptability than TG and TY. Njoya *et al*. (2018) also attributed decrease in the overall acceptability of yoghurt to increase in the concentration of ginger. Thus, yoghurt with high acceptability can be obtained from blends of dairy and tiger-nut milk with or without ginger extract to reduce cost of production, increase accessibility of yoghurt and improve the nutritional status of populace.

Table 5. Sensory evaluation of yoghurt samples

Samples	Colour	Texture	Flavour	Taste	Overall acceptability
DY	$8.60±1.06^{a}$	$5.00 ±1.62^{d}$	$8.10 ±1.02^{a}$	$8.10±0.99^{a}$	$8.60±1.06^{a}$
TY	$4.30±1.32^{c}$	$4.30 ±1.83^{d}$	$4.40 ±1.41^{c}$	$4.30 ±1.61^{cd}$	$4.30±1.32^{c}$
BY	$5.40±1.34^{b}$	$4.40 ±1.23^{d}$	$5.20 ±1.23^{ab}$	$5.60±1.17^{ab}$	$5.40±1.34^{b}$
DG	$8.30±0.91^{a}$	$5.80±1.27^{c}$	$7.40 ±2.30^{a}$	$6.70±1.05^{b}$	$8.30±0.91^{a}$
TG	$4.00±1.77^{c}$	$7.10±1.26^{a}$	$4.40 ±1.18^{c}$	$4.20±1.04^{d}$	$4.00±1.77^{c}$
BG	$5.00±1.06^{b}$	$6.80 ±1.62^{b}$	$6.00 ±1.02^{b}$	$5.40±0.99^{ab}$	$5.70±1.06^{b}$

DY: Dairy Yoghurt, TY: Tiger-nut Yoghurt, BY: 50% Cow milk + 50% Tiger-nut Yoghurt, DG: Dairy Yoghurt + 4% Ginger Extract, TG: Tiger-nut Yoghurt + 4% Ginger Extract, BG: 50% Cow milk + 50% Tiger-nut Yoghurt + 4% Ginger Extract. Values are means of three replicates ± standard error. Means followed by different superscript in the same row are significantly different at $p<0.05$

CONCLUSION

Ginger extract (4%) increased the shelf life of the yoghurt products for four weeks. All samples with ginger extract remain stable and retained the fresh aroma while samples without ginger extract showed changes in aroma, viscosity and appearance after week 2. Thus, safe yoghurt with extended shelflife can be processed from dairy, tiger-nut milk and their blends. It is recommended that people should be sensitized on the importance of plant milk to enhance good nutrition and healthy living. Awareness should also be raised on importance to tiger-nut yoghurt in the developing countries to increase its acceptability.

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