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Research Article

Microbiological characteristics, proximate composition and sensory properties of plant-based set yoghurt analogue produced from blends of maize-soybean

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Abstract

This research produced yoghurt analogue from vitamin A bio-fortified maize with or without substitution with soymilk to combat micronutrient deficiencies. Microorganisms were enumerated and isolated and pH was also determined at 7 days intervals for three weeks. Proximate composition, organoleptic properties and total sugar were also determined on the products using standard methods. The result showed that the TVC and LAB count was within the range of 4.778-8.193 log CFU.ml^{-1} and 6.000-8.662 log CFU.ml^{-1} respectively and the pH generally decreased during the period of storage at refrigeration temperature. *Streptococcus thermophilus*, *Lactobacillus delbrueckii spp. bulgaricus*, *Bacillus licheniformis*, *Saccharomyces cerevisiae* and *Aspergillus niger* were isolated from the samples during storage. Protein (2.23-2.86%), fat (0.43-0.61%) and ash (0.41-0.56%) contents increased while carbohydrate contents decreased with an increase in the addition of soymilk. There was no significant difference ($p>0.05$) in the scores for colour, sourness and overall acceptability of all samples. The addition of soymilk at 50% significantly increased ($p<0.05$) the taste and aroma of the yoghurt analogue. Thus, cereal-based yoghurt analogue can be produced from maize milk analogue substituted with up to 50% soymilk to enhance the consumption of nutrient-dense plant-based yoghurt analogue.

Keywords
maize, soybean, sensory, proximate composition, yoghurt, lactic acid bacteria

Abbreviations
LAB – lactic acid bacteria count; MRS – de Man Rogosa and Sharpe; NA – Nutrient agar; PDA – potato dextrose agar; TTA – titratable acidity; TVC – total viable count

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Introduction

Yoghurt is one of the most popular fermented dairy products consumed all over the world. It is an important dairy product which supplies essential amino acid, calcium and vitamin D. Yoghurt products include, set yoghurt and stirred yoghurt with different flavours with or without the addition of sucrose. It has a smooth texture, sour taste and pleasant flavour. It is produced by inoculating dairy milk with *Lactobacillus delbrueckii spp. bulgaricus* and *Streptococcus thermophilus.* These microorganisms give yoghurt its desirable sour taste and flavour by producing acetaldehyde (Qureshi et al. 2011; Malomo and Abiose 2020b).

Dairy yoghurt is consumed all over the world but there are few exceptions due to nutritional status, religious reasons and financial status. To ensure consumers' satisfaction and accessibility to good nutrition, dairy yoghurt has been substituted with legume yoghurt analogue with similar nutritional composition, rheological and organoleptic properties. Legumes are an affordable source of protein and micronutrients, especially for those living in low- and middle-income countries where animal proteins are expensive and not affordable to the average citizen (Malomo et al. 2019).

Soybean (*Glycine max*) is a common legume which is available and affordable all over the world. It is a very important source of protein, minerals, fat and vitamin B contributing significantly to the nutritional requirement of people living in developing countries. During fermentation by lactic acid bacteria, beany flavour which has a negative effect on the acceptability of soybean products is reduced and the texture improves resulting in products with high acceptability (Obadina et al. 2013; Malomo and Abiose 2020a; Agim-Ezenwaka et al. 2020).

Cereal is the major source of energy in developing countries and could be used in developing functional foods to improve food security in the region (Kearney 2010). Maize (*Zea mays*) is cultivated globally for its high energy and micronutrient content (Prashanthi et al. 2017). It ranked third after wheat and rice regarding the area of cultivation. It is important in the production of breakfast cereal, fermented dumplings, porridges, composite flour and adjuncts in beer production (Oladejo and Adetunji 2012; Malomo and Abiose 2020).

Yellow maize has been bio-fortified with vitamin A to reduce the problem of micronutrient deficiencies in Africa. International Institute for Tropical Agriculture (IITA) in conjunction with Agricultural Research and Training (IAR and T), Nigeria developed pro-vitamin A maize through a project funded by HarvestPlus. Researchers have confirmed that the two bio-fortified hybrids produced can supply vitamin A when boiled, roasted or processed into flour or fermented (IITA 2012). Maize milk analogue is a new product from maize which has been used in the production of cereal-based yoghurt analogue and has been reported to have good organoleptic and nutritional properties similar to other vegetable-based milk (Yasni and Maulidya 2014). The beverage produced from the combination of cereal and legumes has been reported to provide balanced nutrition (Makanjuola 2012). Many authors have worked on the production of yoghurt from cow milk, soymilk and other legumes but there is still a dearth of information on the microbiological and chemical composition of maize-based yoghurt analogue. Hence, this research aims to study the suitability of maize milk analogue with or without the addition of soymilk in the production of a safe and acceptable cereal-based set yoghurt analogue.

Materials and Methods

Soybean and yellow maize (DMR SLR-yellow) were obtained from the Teaching and Research farm in Obafemi Awolowo University (OAU), Ile-Ife, Nigeria, Yogourment was obtained from OAU central market. All chemicals (Sigma, USA) and media (Lab M) used were of analytical standard.

Preparation of maize and maize-soy yoghurt analogue. Maize was harvested and the grains were removed from the cob, cleaned and wet milled in the blender. The slurry was thereafter screened through muslin cloth to obtain the milk analogue. The milk analogue obtained was dispensed into a sterile container for further use. Soybean was sorted, washed and steeped in portable water for 12 h at room temperature. Steep water was drained and boiled for 20 min in water before dehulling. The dehulled beans were milled, homogenized with water, and sieved with muslin cloth and the residue was discarded. Maize milk analogue and soymilk were mixed at ratios 80: 20; 70: 30; 60: 40; and 50: 50 while 100% maize milk analogue served as the control. The samples were inoculated with 1.5% of commercial yoghurt culture containing *Lactobacillus delbrueckii spp. bulgaricus* and *Streptococcus thermophilus* and incubated for 12 h at 45℃. The yoghurt analogue samples obtained were thereafter stored in the refrigerator for further analysis (Omueti and Ajomale 2005).

Microbial analysis. Each sample of yoghurt analogue (5 ml) was dispensed into a stomacher bag containing peptone water (45 ml) and homogenized in a stomacher. The mixture obtained was diluted appropriately and dispensed into the sterile petri dish. Molten agar (20 ml) was poured and rocked gently for an even distribution of growth (Malomo and Abiose 2020a). NA, MRS and PDA are used for total viable count (TVC), Lactic acid bacteria count (LAB) and fungi count respectively and plates were incubated anaerobically for LAB count at 35℃ for 72 h in an inverted position, TVC at 35℃ for 24 h and fungi at 25℃ for 3 to 5 d. The enumeration of microorganisms was determined in triplicate for each yoghurt analogue sample. Colonies formed were counted and steaked to obtain pure culture. Pure bacteria isolates obtained were identified using cultural and morphological characteristics, reaction to Gram's staining and biochemical tests using the scheme described by Harrigan (1998) and Wood and Holzapfel and Wood (2012). Yeast isolates were identified using cultural characteristics, shape, size, and types of budding using a microscope (Leica DM500 Model 13613210), and nitrate and carbon assimilation were also assessed. Mould was identified using colour, type of spores, mode reproduction and presence of special structures (Barnett et al. 2000).

Determination of the proximate composition. Crude protein content was determined using a Kjedhal analyzer, fat content was determined by Soxhlet extraction, ash content was determined using a muffle furnace and the carbohydrate content of maize and maize-soy yoghurt analogue was determined by difference using the method of AOAC (2005b,c,d). The proximate composition of all yoghurt analogues produced was determined in triplicate.

Determination of pH. pH of yoghurt analogue samples was determined using a digital pH meter (Philips model PHS-3C). The pH meter was calibrated with buffer solutions of pH 4 and pH 7. Each yoghurt analogue (10 ml) was dispensed into a conical flask, pH was read in duplicate and the average was calculated AOAC (2005a).

Determination of total sugar. The total sugar content of yoghurt analogue samples was determined by Morris (1948). Each sample was filtered with Whatman 1 filter paper and the filtrate (1 ml) was dispensed into a test tube. Anthrone reagent (4 ml) was added and heated in a boiling water bath (Gallenkomp, HH-S6, England) for 10 min and cooled. The absorbance was read in a UV-spectrophotometer (Spectrumlab 752S, YM1206PHB2, China) at 620 nm. The amount of total sugar liberated was extrapolated from the standard curve of known concentrations of glucose (10-100 $mg.l^{-1}$). (Malomo et al. 2022)

Sensory evaluation. The maize-soy yoghurt analogue samples were coded and presented to selected semi-trained Judges who were familiar with yoghurt for evaluation of colour, aroma, mouthfeel, taste and overall acceptability using a seven-point Hedonic scale where 1 indicated dislike extremely and 7 like extremely (Montgomery 2004).

Statistical analysis. Data obtained from the experiments were evaluated using Analysis of Variance and means were separated using Duncan Test on SPSS 2010. XLSTAT 2018 was used for panel analysis.

Results and Discussion

Changes in the microbial count of maize and maize-soy yoghurt analogue. The total viable count and lactic acid bacteria count of the maize-soy yoghurt analogue are shown in Fig. 1 and Fig. 2. The TVC of maize-soy yoghurt analogue increased with an increase in the days of storage (4.778–- 8.193 log $CFU.ml^{-1}$). The lowest count was observed in 100% maize yoghurt (4.778 log $CFU.ml^{-1}$) while the highest count was recorded in yoghurt analogue produced from 60% maize and 40% soymilk (5.898 log $CFU.ml^{-1}$) at the beginning of storage. The count was generally higher in samples containing soybean than 100% maize from day zero to day 14 probably due to an increase in the availability of amino acids and other nutrients with the addition of soybeans (Malomo et al. 2019).

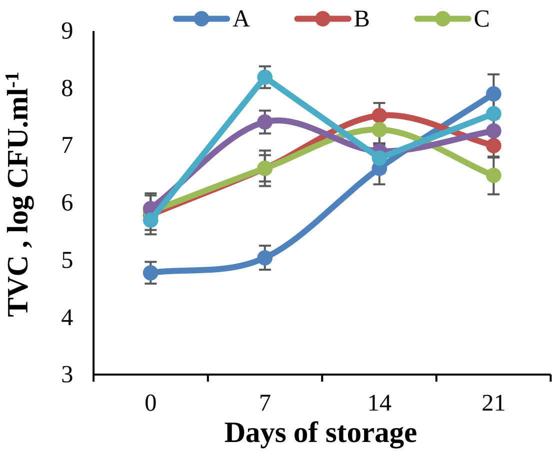

Figure 1. TVC of maize-soy yoghurt during storage

A: 100% maize yoghurt analogue; B: 80% maize and 20% soy yoghurt analogue; C: 70% maize and 30% soy yoghurt analogue; D: 60% maize and 40% soy yoghurt analogue; E: 50% maize and 50% soy yoghurt analogue

The LAB count range in maize and maize-soy yoghurt analogue was between 6.000 and 8.662 log CFU.ml^{-1} during the period of storage.

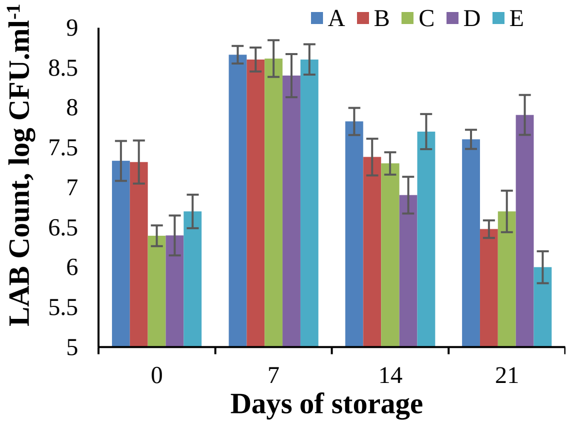

Figure 2. Lactic acid bacteria count of maize-soy yoghurt analogue.

A: 100% maize yoghurt analogue; B: 80% maize and 20% soy yoghurt analogue; C: 70% maize and 30% soy yoghurt analogue; D: 60% maize and 40% soy yoghurt analogue; E: 50% maize and 50% soy yoghurt analogue

Count was highest in the 100% maize yoghurt analogue from day 0 to day 14 with the range of 7.332 to 8.662 log CFU.ml^{-1}. It was highest in yoghurt analogue produced from 100% maize probably because of the high pH that was suitable for the growth and reproduction of *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus*. Counts in all yoghurt analogue samples show that maize-soy yoghurt analogue can be consumed as a functional drink because it contains the permissible quantity of lactic acid bacteria recommended by standard organizations (Codex Alimentarius 2011).

Microorganisms isolated from maize and maize-soy yoghurt analogue. Microorganisms isolated are *Streptococcus thermophilus, Lactobacillus delbrueckii spp. bulgaricus, Bacillus licheniformis, Saccharomyces cerevisiae* and *Aspergillus niger. Streptococcus thermophilus* is a gram-positive cocci shaped microorganism with a diameter ranging from 0.7–1.0 µm. They are facultative anaerobes that grow optimally at 45℃ and can also withstand a temperature of 65℃ for 30 min. They occur in pairs and long chains (De Vos, 2009). They accounted for about 42% of the microorganisms isolated from the samples. *Bacillus licheniformis* is a gram-positive rod with an endospore which could be centrally or subterminal located (De Vos, 2009; Makowski et al. 2021). *Lactobacillus delbrueckii spp. bulgaricus* are gram-positive rods, which occur singly or in chains. *Lactobacillus delbrueckii spp. bulgaricus* accounted for about 37% of the total microorganisms isolated probably because of the use of yoghurt starter culture. Both *Streptococcus thermophilus and Lactobacillus delbrueckii spp. bulgaricus* (Fig. 3a and 3b) were present in the samples from day 0 to day 21. *B. licheniformis* has been reported as a probiotic microorganism that can adapt to the human gastrointestinal tract (Muras et al. 2021; Ramirez-Olea et al. 2022). It was isolated from all yoghurt containing soymilk from day 14 to day 21. *Saccharomyces cerevisiae* (Fig. 3c) is the most common yeast in the fermentation of plants (Malomo et al. 2018). It was isolated in 100% maize yoghurt from day 14 to 21 and in all other samples on day 21. *Aspergillus niger* was isolated in yoghurt produced from 100% maize at day 21.

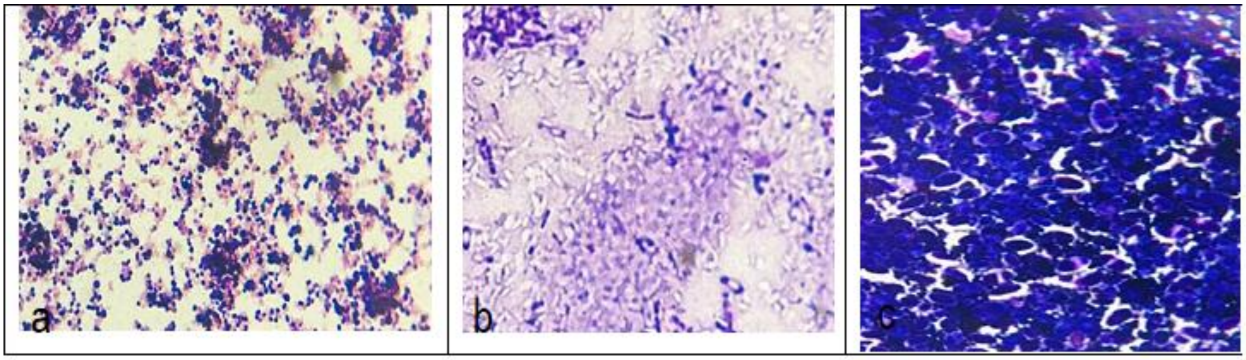

Figure 3. Pictures of bacteria and yeast isolated from maize-soy yoghurt (X100 magnification)
a. *Streptococcus thermophilus;* b. *Lactobacillus delbrueckii spp. bulgaricus;* c. *Saccharomyces cerevisiae*

Proximate composition of maize and maize-soy yoghurt analogue. The proximate composition of maize and maize-soy yoghurt analogue is shown in Table 1. The moisture content was within the range of 90.70 to 91.43%. It was highest in the sample produced from 100% maize (91.43%) and lowest in the yoghurt analogue sample produced from 50% maize milk analogue and 50% soymilk (90.70%). The reduction could be due to the high amount of protein in soybean which increased the water binding capacity of the maize-soy yoghurt analogue samples. The values obtained were similar to 90.61-93.90 reported by Ejinkeonye and Fabian (2018) for soybean yoghurt.

Table 1. Proximate composition of maize-soy yoghurt analogue

	Moisture, %	**Protein, %**	**Fat, %**	**Ash, %**	**Carbohydrate, %**
A	91.43± 0.28^{a}	2.23± 0.02^{e}	0.43± 0.28^{d}	0.41± 0.03^{c}	5.51± 0.11^{a}
B	91.23± 0.35^{ab}	2.37± 0.02^{d}	0.48± 0.11^{cd}	0.43± 0.02^{c}	5.47± 0.10^{a}
C	91.15± 0.28^{b}	2.53± 0.03^{c}	0.52± 0.01^{bc}	0.50± 0.01^{b}	5.33± 0.07^{a}
D	90.85± 0.21^{c}	2.75± 0.01^{b}	0.56± 0.01^{ab}	0.55± 0.07^{ab}	5.31± 0.06^{a}
E	90.70± 0.21^{c}	2.86± 0.04^{a}	0.61± 0.03^{a}	0.56± 0.07^{a}	5.30± 0.07^{a}

A: 100% maize yoghurt analogue; B: 80% maize and 20% soy yoghurt analogue; C: 70% maize and 30% soy yoghurt analogue; D: 60% maize and 40% soy yoghurt analogue; E: 50% maize and 50% soy yoghurt analogue. Superscript on mean values on each column indicate significant difference at $p<0.05$

The protein content of maize and maize-soy yoghurt analogue was between 2.23 to 2.86%. It significantly increased ($p<0.05$) in all samples with an increase in the concentration of soymilk. Soybean contains about 40% protein while maize is low in protein content. This is an indication that the soybeans caused an increase in the protein content of the yoghurt. Studies have shown that the addition of soybeans increases the protein content of cereals, roots and tuber (Owuzu-Kwarteng et al. 2014). Malomo et al. (2019) also observed an increase in the free amino acid content of *masa* snacks with an increase in the concentration of soybeans. The protein content of the 100% maize yoghurt analogue sample and all maize-soy yoghurt analogue samples was higher than the value reported by Descalzo et al. (2018).

Maize and maize-soy yoghurt analogues had fat content ranging from 0.43-0.61% with sample E having the highest percentage. The fat content was significantly higher ($p<0.05$) in samples C, D and E than in sample A. The increase observed was a result of the increase in the proportion of soybean milk which is an indication that soybeans are high in fat. The values obtained in this study are lower than 0.79-2.35% reported by Ejinkeonye and Fabian (2018) and this is due to the difference in the yoghurt constituents. There was no detectable crude fibre in all the yoghurt-like samples and this also supports the findings on soybean yoghurt reported by Ejinkeonye and Fabian (2018).

The ash content was significantly ($p<0.05$) highest (0.56%) in sample E yoghurt and lowest in sample A (0.41). Ash content is an indication of how rich the mineral content in a product is. The increase in ash content was observed among the samples as the quantity of soymilk increased and this is because the soybean seeds have been reported to contain an appreciable quantity of minerals (Sanful and Darko 2010). A decrease in the carbohydrate was observed with an increase in concentration of soymilk but it was not significant ($p<0.05$).

The total sugar content of maize and maize-soy yoghurt analogue. The total sugar of maize-soy yoghurt is shown in Fig. 4. It was highest in 100% maize yoghurt analogue (22.9 mg.ml^{-1}) and reduced with an increase in the addition of soymilk at day zero. Maize-soy yoghurt analogue produced from 100% maize also had the highest concentration of total sugar at days zero, fourteen and twenty-one.

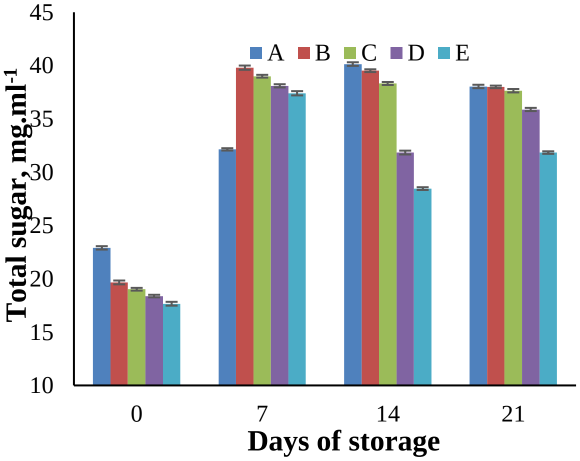

Figure 4. Total sugar content of maize-soy yoghurt.
A: 100% maize yoghurt analogue; B: 80% maize and 20% soy yoghurt analogue; C: 70% maize and 30% soy yoghurt analogue; D: 60% maize and 40% soy yoghurt analogue; E: 50% maize and 50% soy yoghurt analogue

The total sugar generally reduces with an increase in the concentration of soymilk throughout the period of storage. This may be due to the low carbohydrate content of soymilk and the high carbohydrate content of maize. It has been reported that carbohydrate is broken down into simple sugars by lactic acid bacteria and other microorganisms involved in fermentation (Adepoju et al. 2016). According to Jideani and Jideani (2011), the high level of total sugar in 100% maize yoghurt analogue could be attributed to the high level of digestible carbohydrates which were broken down into simple sugar by the microorganisms involved in the fermentation process.

pH of maize and maize-soy yoghurt analogue during storage. The pH of the yoghurt analogue samples is shown in Fig. 5. pH ranged between 3.90 and 5.34 during the period of storage.

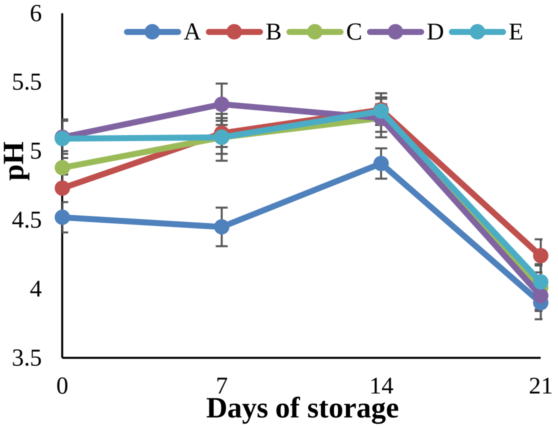

Figure 5. The pH of maize-soy yoghurt during storage.
A: 100% maize yoghurt analogue; B: 80% maize and 20% soy yoghurt analogue; C: 70% maize and 30% soy yoghurt analogue; D: 60% maize and 40% soy yoghurt analogue; E: 50% maize and 50% soy yoghurt analogue

Yoghurt containing 100% maize had the lowest pH throughout the period of storage. The pH generally increased with an increase in the addition of soymilk. A similar observation was reported by Malomo et al. (2018) during the production of masa. Low pH in 100% maize yoghurt could be attributed to the acidic nature of Zein protein in maize. Also, acid production depends on the ability of microorganisms to metabolize carbohydrates which is more abundant in maize than soybeans (Makunjuola 2012).

Sensory properties of maize and maize-soy yoghurt analogue. Maize-soy yoghurt containing 50% maize and 50% soy had the highest score for colour (5.51), aroma (5.00), sourness (5.00), taste (5.40) and overall acceptability (5.00) (Table 2). There was no significant difference ($p>0.05$) in the scores for colour, sourness and overall acceptability of all samples but the scores for aroma and taste of sample E was significantly higher ($p<0.05$) than other samples. Adding soymilk had a significant effect ($p<0.05$) on the taste and aroma of maize-soy yoghurt analogue at 50% concentration. Low scores recorded for the samples could be a result of the absence of sucrose.

Table 2. Sensory properties of maize and maize-soy yoghurt analogue

	Colour	Aroma	Sourness	Taste	Overall acceptability
A	4.60±1.77^{a}	3.10±1.29^{b}	3.60±2.05^{a}	2.80±1.03^{b}	3.60±1.78^{a}
B	4.40±1.42^{a}	3.20±1.03^{b}	4.50±1.71^{a}	3.20±1.61^{b}	3.70±1.25^{a}
C	5.50±0.71^{a}	3.30±0.94^{b}	4.50±1.58^{a}	3.40±1.58^{b}	3.90±1.41^{ab}
D	5.50±0.71^{a}	3.80±1.26^{b}	4.50±1.40^{a}	3.50±0.84^{b}	4.30±0.99^{ab}
E	5.51±0.53^{a}	5.00±0.86^{a}	5.00±1.32^{a}	5.40±1.07^{a}	5.00±1.05^{a}

A: 100% maize yoghurt analogue; B: 80% maize and 20% soy yoghurt analogue; C: 70% maize and 30% soy yoghurt analogue; D: 60% maize and 40% soy yoghurt analogue; E: 50% maize and 50% soy yoghurt analogue. Superscript on mean values on each column indicate significant difference at $p<0.05$

Panel analysis. The response of the assessors on aroma showed that assessors 3, 5, 8, 9, and 10 had similar observations, assessors 6 and 7 also had similar observations but assessor 2 response was different from the response of other assessors (Fig. 6). Assessors 2, 3 and 4 had similar observations of the colour, 1, 6, 7, 8, 9 and 10 also had similar observations while assessor 5 view on the colour was different from other assessors showing the farthest distance. All assessors had similar responses for taste which all clustered at the outer circle. For sourness, the responses of accessors 2, 3, 4, 5 and 10 clustered together, 1, 6, 7 and 8 also clustered together but the response of assessor 9 has the farthest distance from all other responses. All assessors except 5 had similar observations for the overall acceptability of the yoghurt analogue. This shows that the assessors are familiar with yoghurt because only 2, 5 and 9 had a different response on the aroma, colour, overall acceptability and taste respectively. Showing that about 70% of the assessors have similar responses on the maize and maize-soy yoghurt analogue.

Conclusions

The lactic acid bacteria count of all maize-soy yoghurt samples produced was within the permissible limit during the period of storage. The higher percentage of lactic acid bacteria isolated from maize-soy yoghurt analogue shows that it is a potential functional protein-energy drink that can provide adequate balance nutrients for the teaming population. The result of the organoleptic evaluation shows that the yoghurt analogue produced from 50% maize and 50% soymilk was most preferred. Thus, safe yoghurt analogue with adequate nutritional quality can be produced from maize milk and soymilk.

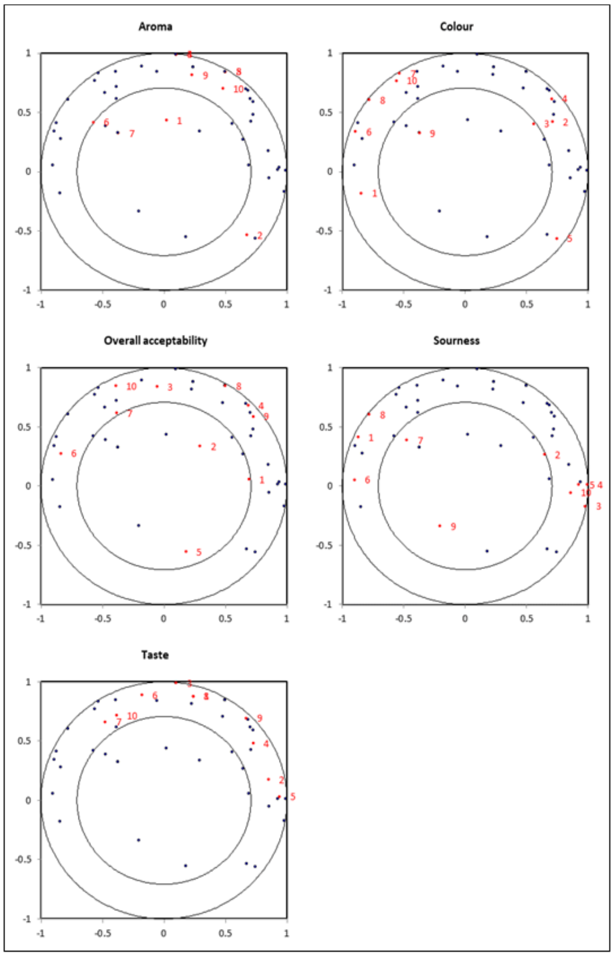

Figure 6. Responses of the taste panelists

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