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## The Effect of Varying Starter Culture Content on the Characteristics of Peanut Yogurt Produced from Peanut Milk

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### **Abstract:**

Presently, a considerable number of people go for plant milk substitutes due to various reasons such as medical reasons or lifestyle choices. Medical reasons include lactose intolerance, cow's milk allergy and cholesterol issues. Life style choices such as vegetarian or vegandiet concerns about growth hormones or antibiotic residues in cow's milk also influence these choices. The possibility of producing fermented peanut beverage (Peanut yoghurt) from lactose free milk-like beverage (peanut milk) was studied. Plant milk substitutes (such as peanut milk) are suspensions of dissolved and disintegrated plant materials in water resembling cow's milk. Peanut yogurt with varying amount of starter culture was successfully produced by fermenting peanut milk. The increase in starter culture content was done in an attempt to reduce the nutty flavour which is an undesirable characteristic in the peanut milk product. The peanut yogurt improved gradually as the starter culture content increased. The peanut yogurt was subjected to physicochemical, proximate, micro biological analysis and sensory evaluation. The peanut yogurt with the highest starter culture content was discovered to have the highest viscosity value and better aroma than the other variations, it also had low fat content and high protein and carbohydrate content. Sensory evaluation of peanut yoghurt using the nine-point hedonic scale with the help of fifteen panelists showed that as the starter culture increased peanut yogurt with acceptable quality attributes was achieved. Therefore, peanut yogurt can be a successful alternative to milk yoghurt manufacture in regions with high peanut production.

**Keywords:** Peanut, peanut milk, fermentation, yogurt, acceptability

### **1. Introduction**

Due to the incidences of lactose intolerance and other health issues related to consumption of milk such as cholesterol issues, and life style choices (vegetarian diet), concerns about antibiotic residues in cow's milk, an alternative source of milk is required. However, since milk production is mainly attributed to animals, products from plants which resembles milk can be referred to as imitation milk. Some sources of plant imitation milk include milk like substances from peanut, almond, soy, and rice.

Milk is referred to as a complete food, having all the nutrients required for effective growth and development (Habtamu et al., 2015). Peanuts are also rich in essential nutrients therefore it could serve as an alternative to milk, providing comparable nutrients as found in milk for people who cannot consume milk for various reasons. Peanuts are a rich source of proteins and essential amino acids (Pelto & Armar-Klemesu, 2011) and also contain lipids and carbohydrates which are energy rich compounds capable of complementing the basic energy demands of the human body (Settaluri et al., 2012). In a 100g serving, dry-roasted peanuts provide energy (585kcal) water (1.6g), carbohydrates (21.5g), fiber (8.0g), fats (49.7g), proteins (23.7g) (USDA, 2011).

Peanut milk is a non-dairy beverage created using peanuts and water. Recipe variations include salt, sweeteners, and grains. Similar in production to almond milk, soy milk, and rice milk, the peanuts are typically ground, soaked, sometimes heated, and then filtered through a fine filter (muslin cloth), the resulting liquid is considered the "milk", which could also be referred to as 'imitation milk'. Imitation milk has been used to describe products that have the characteristics of milk but do not have any form of milk fat nor dairy ingredient (Potter & Hotchkiss, 1996). It does not contain any lactose and is therefore suitable for people with Lactose intolerance. Lactose intolerance is a condition in which people have symptoms due to the decreased ability to digest lactose, a sugar found in milk products into its constituents such as glucose and galactose due to absence or low levels of lactase enzyme (Rusnyk & Still, 2001). Symptoms may include abdominal pain, bloating, diarrhea, gas and nausea.

In the production of peanut milk, immense effort is being paid to the improvement of the stability, sensory properties, and shelf life of the peanut milk, using physical and chemical treatments. However, despite all these

developments, there is still a need for more studies in order to overcome the nutty flavour and sensory problems encountered when producing peanut milk and some peanut milk-based products. Fermentation could be the answer to these problems. According to Beuchat (1995), fermentation is in fact expected to cause major changes in odor, aroma, flavour and texture of food. Therefore, this study focused on improving the quality of peanut milk by fermentation into a yoghurt like beverage using varying amount of starter culture to determine its effect on the quality characteristics of the peanut yoghurt like beverage.

## 2. Materials and Methods

### 2.1. Materials

The raw peanuts used in this study were purchased from a local market in Ikotun, Lagos state, Nigeria. The yoghurt starter cultures (*Lactobacillus Bulgaricus*, *Lactobacillus acidophilus* and *Streptococcus thermophilus*) were purchased from a supermarket in Lagos state, Nigeria. All chemicals and reagents used were of food grade.

### 2.2. Methods

#### 2.2.1 Peanut Milk Preparation

Peanut milk was prepared according to the method described by Ojofeitimi et al. (2001). The peanuts were sorted to separate the foreign materials that could affect the properties of the product from the lot, then they were soaked in a generous amount of cool water for some hours. After soaking, the peanuts were blended with water till smooth, then sieved with a muslin cloth, the milk was poured into a pot.

#### 2.2.2 Preparation of Peanut Milk Yogurt

Peanut milk yoghurt samples were prepared according to the method described by Chan & Beuchat (1992). The peanut milk was pasteurized at 185°F (85°C) for 30 minutes. The milk was then cooled to 108°F (42°C) to bring the milk to the ideal growth temperature for the starter culture. The starter cultures were mixed into the cooled milk and left to ferment. The yogurt was then cooled to 7°C to stop the fermentation process. Yogurt samples were prepared by varying the starter culture content (2.5g, 5.0g, 7.5g, 10.0g) in the peanut yogurt. Peanut milk was used as control.

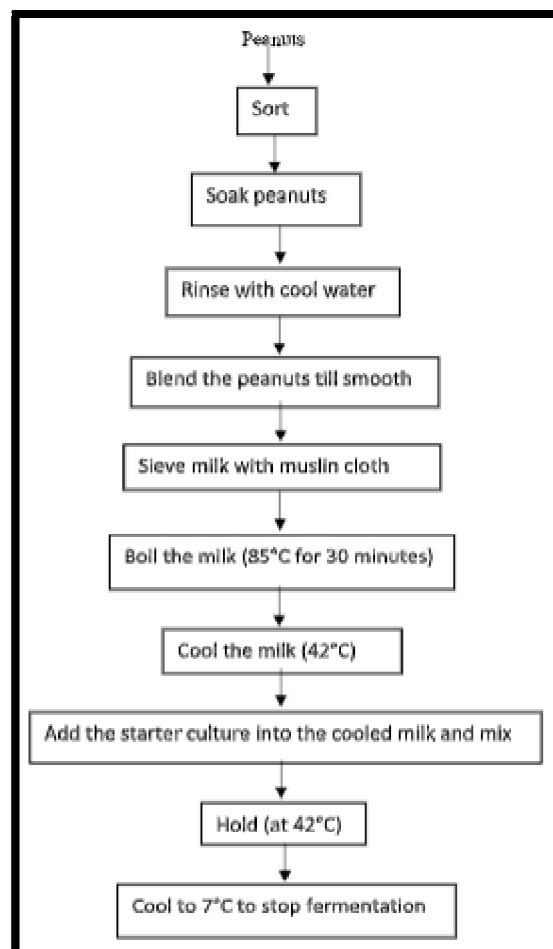


Figure 1: Flow Chart for the Production of Peanut Milk Yogurt

### 2.2.3 Physio-Chemical Analysis

#### 2.2.3.1.pH

The pH value of the peanut samples was measured using the method described by AOAC (2006). A digital pH meter was calibrated with standard buffers (pH 4.0 and 7.0) and then used to take the reading. The results were obtained and recorded.

#### 2.2.3.2. Total Titratable Acid

The total titratable acidity of peanut yogurt sample was determined using a previously reported procedure by AOAC (2006). Ten millilitres of yogurt sample were mixed with 10ml of distilled water and then transferred to a conical flask using a 10ml pipette and titrated against 0.1M sodium hydroxide (NaOH) solution using a 1% phenolphthalein indicator to an end point of faint pink colour. The result was calculated as follows:

$$\frac{\text{Titre value} \times \text{molarity} \times 0.09 \times 100}{\text{Volume of sample (ml)} \times 1}$$

### 2.2.4. Proximate Analysis

#### 2.2.4.1. Determination of Ash Content

Ash content was determined as described by the AOAC(1995) method. About 2g of the sample was weighed into a crucible of known weight. The content in the crucible was pre-ashed evenly using a hot plate until the smoking stopped. At about 500°C to 570°C, it was put in a muffle furnace for about six hours until the white or grey ash was gotten. Using a crucible tong, the crucibles were transferred into a desiccator and allowed to cool. The crucible and the ash were reweighed (Wc). The percentage ash content was calculated thus:

$$\% \text{ Ash content} = \frac{W_c - W_a}{W_b - W_a} \times \frac{100}{1}$$

Where Wa is weight of empty crucible

Wb is weight of sample with crucible before ashing

Wc is weight of sample with crucible after ashing

#### 2.2.4.2. Determination of Fat Content

About 5g of sample was weighed and transferred to an extraction tube, 2ml of ammonia was added and mixed thoroughly. 10ml of ethyl alcohol was added and mixed again. From a mixture of 50ml of diethyl ether and 50ml of petroleum ether, 25ml was taken and added to the solution and mixed vigorously each time to release the gas until no gas was produced again. It was left to stand until the upper ethereal layer was separated and completely clear (alternatively, a centrifuge was used to separate the layers). A pipette was then used to collect the top which contained the extracted fat into a conical flask of known weight. The conical flask containing the extracted fat was placed on a water bath to evaporate the solvents (petroleum ether and diethyl ether), it was allowed to evaporate off completely. The beaker was dried in an oven for 5mins at 100°C, cooled in a desiccator and weighed.

Calculation;

$$\text{Fat (\%)} = \frac{\text{Weight of beaker after drying} - \text{Weight of empty beaker}}{\text{Weight of sample}} \times 100$$

#### 2.2.4.3. Determination of Protein Content

The protein was determined using Kjeldahl distillation method as described by AOAC(2000). 0.5ml of the sample was weighed and transferred to the kjeldahl digestion flask and 5g of catalyst added with 20ml of concentrated H<sub>2</sub>SO<sub>4</sub>. The flasks were then placed in an inclined position on the heating mantle and heated gently until the frothing ceased and briskly boiled until the liquid became clear. A portion of the sample will be converted to ammonia acid and ammonia sulphate during digestion. The digest was diluted by adding distilled water cautiously to make it up to 50ml. The flask was connected to the digestion bulb in the condenser and the tip of the condenser immersed in standard acid and 10ml of indicator in a conical flask (the receiver), the flask was rotated to mix content and then heated till all NH<sub>3</sub> was distilled. The ammonia will be distilled into 2% boric acid (50ml) and the condenser removed as well as the delivery tube after washing into the receiver. The distillate will be titrated with 0.1N HCL to purplish grey end point.

The percentage protein (%P) will be calculated thus:

$$\text{Nitrogen Content} = \frac{((\text{ml})\text{HCL} - (\text{ml})\text{blank}) \times N \times 14.01}{W}$$

$$\% \text{ Crude protein} = 6.25 \times \text{nitrogen Content}$$

Where;

N is normality of HCL

W is weight (g) of sample

14.01 is the atomic weight of Nitrogen

6.25 is the protein-nitrogen conversion factor

#### 2.2.4.4. Determination of Moisture Content

The moisture content was determined using the oven method described by AOAC(2000). The moisture cans were washed and dried in the oven and weighed using analytical weighing balance. 3g of the sample was put into the previously

weighed moisture cans. The samples in the moisture were put into the oven at 105°C for 3hours then removed and placed in a desiccator to cool, after cooling it was weighed.

$$\text{Moisture content (\%)} = \frac{(W_1 - W_2) \times 100}{W_1 - W}$$

Where;

W1 is weight (g) of sample before drying

W2 is weight (g) of sample after drying

W is weight (g) of the empty dish

#### 2.2.4.5. Determination of Carbohydrate Content

The carbohydrate content of the yogurt was determined by difference according to AOAC(1995). The percentage of moisture, protein, fat and ash is subtracted from 100.

$$\%CHO = [100 - (M.C + \text{protein} + \text{fat} + \text{ash})]$$

#### 2.2.5. Microbial Load

The methods described by AOAC (2006) were used for total viable and fungi count. Approximately 1ml of the sample and 9ml of peptone water was used for microbial analysis and further serially diluted up to 10<sup>-6</sup>. About 1ml of each dilution was discharged into the center of the sterile Petri dishes. Molten Nutrient (NA) agar for total viable count and Sabouraud's dextrose (SDA) agar for fungi count was poured into each Petri dish in duplicate. The plates were allowed to cool and set, and then, incubated by inverting them at 37°C for 24 hours for bacteria enumeration and at 28°C for 72h for fungi. The number of colonies per plate were multiplied by the dilution factor to obtain the viable counts per ml of the original sample and only plates containing between 30 – 300 colonies were counted. The results were expressed as colony forming unit per ml (CFU/ml).

#### 2.2.6. Determination of Viscosity

The viscosity readings were obtained using a viscometer. After calibration, a spindle was attached (A1, A2, A) and samples were transferred to clean beakers up to the calibrated mark, the results were obtained and recorded.

#### 2.2.7. Sensory Analysis

A fifteen-member panel was used to evaluate the various sensory parameters (aroma, appearance, colour, taste, texture, consistency and overall acceptability) after overnight storage at 4-5°C. The score was based on a hedonic scale range from 9 representing "like extremely" to 1 representing "dislike extremely". The samples were presented to the panellists in cups with three random digit labels containing approximately 25ml of sample per cup.

#### 2.2.8. Statistical Analysis

All data were subjected to statistical analysis using a computer program, SPSS system for windows for analysis of variance (ANOVA) by one way and comparison of means by Turkey's multiple comparison test where p<0.05 was considered for significant difference.

### 3. Results and Discussion

#### 3.1. pH and Total Titratable Acidity of the Different Yogurt Samples

The pH levels of the peanut yogurt reduced gradually from 5.64 to 4.86 as the starter culture content increased while the total titratable acidity increased as the starter culture content increased (0.09 to 0.11) (Table 1). pH is measured based on the acid concentration and the amount of dissociated hydrogen ions in the solution while the total acidity measures all hydrogen ions (Tamime and Robinson, 1999). During fermentation, microorganisms use sugars for their metabolic activity and in the process secrete acids as their by-product. According to Willey et al. (2008), microorganisms frequently change the pH of their own habitat by producing acidic or basic metabolic waste product. Therefore, acidity and pH changes could be attributed to the number and or rate of metabolic activity of acid producing micro-organisms. As the starter culture increased, more microorganisms were probably available for metabolic activities thereby producing more acids. Acid production in the medium depends on the growth of micro-organisms and their ability to ferment the available sugars.

#### 3.2. Moisture Content and Total Solid Content of the Different Yogurt Samples

The moisture content of the peanut milk and peanut yoghurt samples are shown in Table 2. Although the moisture content of peanut yogurt that contained 2.5g was higher than the other peanut samples, it was not significantly different from all the other samples at p<0.05 level. The total solid content of peanut samples was also not significantly different from each other at p<0.05 level. The total solid content in the peanut yogurts ranged from 20% to 27%. This is within the range of solids in milk (9% to as high as 30%) for the manufacture of yogurt as reported by Tamime and Robinson (1999).

Samples	pH	TTA
Peanutmilk	6.83 ± 0.02 <sup>a</sup>	0.04 ± 0.00 <sup>d</sup>
Peanutyogurt 2.5g	5.64 ± 0.01 <sup>b</sup>	0.09 ± 0.00 <sup>c</sup>
Peanutyogurt 5.0g	5.42 ± 0.02 <sup>c</sup>	0.09 ± 0.00 <sup>c</sup>
Peanutyogurt 7.5g	5.34 ± 0.01 <sup>d</sup>	0.10 ± 0.00 <sup>b</sup>
Peanutyogurt 10g	4.86 ± 0.02 <sup>e</sup>	0.11 ± 0.00 <sup>a</sup>

Table 1: Ph and Total Titratable Acidity Values of the Different Yogurt Samples

Results are reported as means ± standard deviation. Means bearing different letter(s) in a column are significantly different at level of  $p < 0.05$ .

Samples	Moisture	Total solids
Peanutmilk	72.50 ± 9.01 <sup>a</sup>	27.50 ± 9.01 <sup>b</sup>
Peanutyogurt 2.5g	79.17 ± 1.44 <sup>a</sup>	20.83 ± 1.44 <sup>b</sup>
Peanutyogurt 5.0g	77.50 ± 0.00 <sup>a</sup>	22.50 ± 0.00 <sup>b</sup>
Peanutyogurt 7.5g	78.33 ± 1.44 <sup>a</sup>	21.67 ± 1.44 <sup>b</sup>
Peanutyogurt 10g	74.17 ± 1.44 <sup>a</sup>	25.83 ± 1.44 <sup>b</sup>

Table 2: Mean Moisture Content and Total Solid Content Values of the Different Yogurt Samples

Results are reported as means ± standard deviation. Means bearing different letter(s) in a column are significantly different at level of  $p < 0.05$ .

### 3.3. Proximate Analysis of the Different Yogurt Samples

The results of the proximate analysis of the samples are presented in Table 3. The crude protein content of all peanut yogurt samples increased as the starter culture increased. All the samples were significantly different from each other at  $p < 0.05$  level, this shows that some degree of activities must have occurred during fermentation of milk to yoghurt by the yoghurt starter cultures causing a significant degree of proteolysis (Tamime and Robinson, 1999). According to Thomas and Mills, (1981), the protein content in yogurt depends on the proteolytic activity of the bacteria which hydrolyses proteins (caseins) into peptides and amino acids. The fat content of all peanut yogurt samples decreased as the starter culture increased. All the samples were significantly different from each other at  $p < 0.05$  level. This significant decrease could be due to the presence of more microorganism as a result of increased starter culture hydrolysing the fat in the peanut yoghurt. This agrees with the suggestion of Tamime and Robinson (1999) who suggested that reduction in fat content may be attributed to lipid metabolism by the starter culture micro-organisms and the process of homogenization. Peanut yogurt that contained 5.0g starter culture had the lowest carbohydrate content while peanut yoghurt having the highest starter culture had the highest carbohydrate content. All peanut products were slightly different from each other but were not significantly different at  $p < 0.05$ .

### 3.4. Viscosity of the Different Yogurt Samples.

All the samples were significantly different from each other at  $p < 0.05$  level. The viscosity of the samples increased with increase in the starter culture content. Peanut yogurt containing 10g starter culture had the highest value while peanut milk had the lowest value. This could be because there was no starter culture in the milk. The highest viscosity reading indicates that the starter culture had an effect on the texture of yogurt during coagulation. Coagulation of fermented milk is due to casein protein content. Low viscosity readings may be due to changes in the microstructures of the yogurt curd which became finer and more porous, exhibiting a more continuous casein network composed of smaller particles linked via particle chains (Abrahamsen *et al.*, 1991).

Samples	Ash	Protein	Fat	Carbohydrate
Peanutmilk	3.11 ± 0.38 <sup>a</sup>	5.60 ± 0.03 <sup>a</sup>	5.14 ± 0.12 <sup>c</sup>	13.65 ± 8.68 <sup>b</sup>
Peanut yogurt 2.5g	2.00 ± 0.33 <sup>a</sup>	1.08 ± 0.15 <sup>e</sup>	6.24 ± 0.08 <sup>a</sup>	11.51 ± 1.52 <sup>b</sup>
Peanut yogurt 5.0g	3.33 ± 2.40 <sup>a</sup>	2.25 ± 0.10 <sup>d</sup>	5.55 ± 0.10 <sup>b</sup>	11.37 ± 2.40 <sup>b</sup>
Peanut yogurt 7.5g	4.11 ± 1.54 <sup>a</sup>	2.61 ± 0.12 <sup>c</sup>	2.78 ± 0.07 <sup>d</sup>	12.17 ± 2.55 <sup>b</sup>
Peanut yogurt 10g	3.67 ± 0.00 <sup>a</sup>	2.78 ± 0.07 <sup>c</sup>	1.90 ± 0.07 <sup>e</sup>	17.59 ± 1.58 <sup>b</sup>

Table 3: Mean Proximate Values for the Different Yogurt Samples

Results are reported as means ± standard deviation. Means bearing different letter(s) in a column are significantly different at level of  $p < 0.05$ .

Samples	Viscosity
Peanut milk	68.33 ± 3.75f
Peanutyogurt 2.5g	110.37 ± 2.89e
Peanutyogurt 5.0g	123.87 ± 2.05d
Peanutyogurt 7.5g	210.13 ± 4.84c
Peanutyogurt 10g	363.73 ± 9.20a

Table 4: Mean Values for Viscosity of the Different Yogurt Samples

Results are reported as means ± standard deviation. Means bearing different letter (s) in a column are significantly different at level of  $p < 0.05$ .

### 3.5. Microbial Load

Table 5 shows the number of colonies present in the various samples for fungi count and total viable count. Total viable count and fungi count reduced as the starter culture increased in the peanut yogurt. Increased starter culture probably brought about the production of more organic acids which probably lowered the pH of the peanut milk making it unfavourable for some microorganisms to thrive, thereby leading to lower microbial load in the peanut yoghurt. According to Gahan et al. (1996) accumulation of some inhibitory metabolites such as organic acids produced during fermentation may reduce microbial load. However, the peanut yoghurt with the highest starter culture had the highest total viable count while the lowest count was found in peanut milk. Addition of starter culture to peanut milk may have also probably introduced more microorganisms and also activated microorganisms originally present in the peanut milk thereby increasing the amount of microbes.

Samples	Total Viable Count (PCA)	Fungi Count (SDA)
Peanutyogurt 2.5g	5.1 x 10 <sup>5</sup> CFU/ml	2.4 x 10 <sup>5</sup> CFU/ml
Peanutyogurt 5.0g	5.0 x 10 <sup>5</sup> CFU/ml	2.3 x 10 <sup>5</sup> CFU/ml
Peanutyogurt 7.5g	3.4 x 10 <sup>5</sup> CFU/ml	2.1 x 10 <sup>5</sup> CFU/ml
Peanutyogurt 10g	2.0 x 10 <sup>6</sup> CFU/ml	1.7 x 10 <sup>5</sup> CFU/ml
Peanutmilk	1.2 x 10 <sup>4</sup> CFU/ml	1.2 x 10 <sup>5</sup> CFU/ml

Table 5: Mean Microbial Load Values for the Different Yogurt Samples

### 3.6. Sensory Attributes of Peanut Samples

The result of the sensory evaluation of peanut samples is presented in Table 6. Based on the comments of the panellists, with respect to appearance, there was no significant difference between all the peanut yogurt samples. Peanut yogurt with 2.5g starter culture was the least preferred in terms of taste. This may be due to the be any flavour though reduced but still noticeably in the peanut yoghurt. Although all the peanut yogurt samples were slightly different from each other under Turkey's test, there was no significant difference at  $p < 0.05$  level for taste. In terms of colour, there was no significant difference at  $p < 0.05$  for all the peanut yogurt samples but they were slightly different under Turkey's test. There was no significant difference in the consistency of all the peanut samples at  $p < 0.05$  level but peanut yogurt of 10g starter culture was mostly preferred according to the panellists. However, all the peanut yogurt samples were not significantly different at  $p < 0.05$  level from each other in terms of colour.

Samples	Appearance	Taste	Color	Consistency	Aroma	Texture	Over All Acceptability
Peanutmilk	6.47 ± 1.30 <sup>b</sup>	5.27 ± 2.09 <sup>b</sup>	6.27 ± 1.49 <sup>b</sup>	6.87 ± 1.13 <sup>a</sup>	5.47 ± 1.77 <sup>a</sup>	6.13 ± 1.36 <sup>b</sup>	6.20 ± 2.13 <sup>ab</sup>
Peanutyogurt 2.5g	6.53 ± 0.83 <sup>ab</sup>	4.60 ± 1.80 <sup>b</sup>	6.00 ± 1.41 <sup>b</sup>	6.87 ± 1.00 <sup>a</sup>	5.13 ± 1.46 <sup>a</sup>	6.73 ± 1.03 <sup>ab</sup>	5.93 ± 1.41 <sup>b</sup>
Peanutyogurt 5.0g	6.60 ± 0.83 <sup>ab</sup>	5.40 ± 1.80 <sup>b</sup>	6.13 ± 1.55 <sup>b</sup>	7.07 ± 0.80 <sup>a</sup>	5.47 ± 1.51 <sup>a</sup>	6.53 ± 1.06 <sup>ab</sup>	5.60 ± 1.88 <sup>b</sup>
Peanutyogurt 7.5g	6.67 ± 0.90 <sup>ab</sup>	5.67 ± 2.16 <sup>b</sup>	6.33 ± 1.45 <sup>b</sup>	7.07 ± 0.94 <sup>a</sup>	5.73 ± 1.49 <sup>a</sup>	6.60 ± 1.45 <sup>ab</sup>	5.53 ± 1.61 <sup>b</sup>
Peanutyogurt 10g	6.67 ± 0.90 <sup>ab</sup>	5.67 ± 2.06 <sup>b</sup>	6.20 ± 1.37 <sup>b</sup>	7.20 ± 1.16 <sup>a</sup>	5.47 ± 1.60 <sup>a</sup>	6.40 ± 1.68 <sup>ab</sup>	5.47 ± 1.83 <sup>b</sup>

Table 6: Sensory Attributes of the Different Yogurt Samples

Results are reported as means ± standard deviation. Means bearing different letter (s) in a column are significantly different at level of  $p < 0.05$ .

#### 4. Conclusion

Peanut milk yogurt with acceptable nutritional and sensory characteristics was successfully produced in this study. Though the nutty flavor of peanut milk was drastically reduced as the starter culture increased, it was still an issue. However, peanut yogurt could still be an option to milk yoghurt among consumers and also manufacturers in regions with high peanut production.

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