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Comparative Nutritional Assessment of Yoghurts Produced from Indigenous Plant Materials (*Soybean, Tigernut, Bambara Nut, Black Beans, and Pigeon Peas)*

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Abstract: *Challenges of food security in developing countries has in recent years made researches to be directed towards the development of foods with improved protein quality by the use of legumes which are considered to be nutritionally balanced. In this wise, yoghurts were produced from local plant raw materials including Soybean, Tigernut, Bambara groundnut, Black bean and Pigeon peas (Glycine max, Cyperus esculentus, Vignia subterranea, Vignia ungiculata and Cajanus cajan respectively) which are underutilized loca plants. Commercial Dano milk was also used for the yoghurt as a production control. The work also assessed the effect of using millet steep culture, as a possible replacement for the commercial starter culture which encompasses lactic acid producing bacteria (Lactobacillus bulgaricus and Streptococcus thermophilus). Twelve yoghurt samples were prepared: Dano milk (A), Soybean (B), Tigernut (C), Bambara nut (D), Black bean (E), Pigeon Pea (F), Dano milk (G), Soybean (H), Tigernut (I), Bambara nut (J), Black bean (K), Pigeon pea (L) and Hollandia (M) for comparison (ABCDEF were fermented with commercial starter culture, while GHIJKL were fermented with millet steep culture). They were subjected to proximate, phytochemical, physicochemical, mineral, vitamin, microbial analysis as well as sensory evaluation with a view of understanding the consumer most acceptable product. Commercially acceptable yoghurt brand- plain Hollandia yoghurt was also analysed and used as standard for comparison (sample M). The data generated were analysed using one-way ANOVA followed by Tukey's post hoc test and significant difference set at (p<0.05). The result indicated the presence of alkaloids (0.1 - 0.8mg), flavonoids (0.1 – 1.2mg), saponin (0.1 - 0.4mg), tanins (0.1 – 0.6mg) and oxalate (0.1 - 0.3mg). Vitamins, calcium (0.67 - 45.33mg), potassium (0.23–2.63mg), magnesium (3.98 – 5.88mg), Sulphur (0.0 – 0.2mg) and phosphorus (0.1 – 100.8mg) were at acceptable levels. These and other parameters studied varied significantly (p<0.05) for samples fermented with commercial starter culture, and millet steep culture. The microbial result revealed that total viable count (TVC) ranged from 1.0 x 103 minimum to 3.3 x 103 maximum, pathogenic bacteria (total coliforms and Escherichia coli) were not detected (nil). Accordingly, yoghurt, and yoghurt-like products -were successfully produced from local plant raw materials and there is possibility of using millet steep culture as a substitute for commercial starter culture.*

Keywords: *yoghurt, Nutritional assessment, millet steep and commercial starter culture.*

1. Introduction

Yogurt is one of the most consumed healthy and nutritious foodstuff worldwide (Adriana *et al*, 2018; Shi *et al.*, 2017; Zhi *et al.*, 2018). Yoghurt is "a fermented product obtained by means of anaerobic fermentation of lactose in milk with relevant micro-organisms (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) which are classified as 'probiotic' (friendly, or harmless) microorganisms (Sanful, 2009).

Market reports shows that the yoghurt market is projected to reach at $107,209 million by 2023, which stands for 4.5% growth over 10 years (Prasannan, 2017). Yoghurt is one of the most popular fermented dairy products which have a wide acceptance worldwide. The origin of yoghurt is not well known but believed to be dated back to the 6000 BC when the Neolithic people in the Central Asia transformed from a status of food gatherers to food producers when they began the practice of milking their animals (National Yoghurt Association, 2013). It is generally accepted that the fermented milk products including yoghurts have been discovered accidentally when they used to store milk in sheep-skin bags and unused milk will get sour. Fermentation of milk has therefore evolved over centuries into commercial yoghurt making which paved the way for different commercially available varieties with a range of flavours, forms and textures (National Yoghurt Association,

2013. The composition requirement for milk fat and milk solids non-fat is applied to the yoghurt prior to the addition of bulky flavoring ingredients according to United State Department of Agriculture (USDA, 2016) specifications for yoghurt. Yogurt is considered as healthy food due to its high digestibility and bioavailability of nutrients as recommended to the people with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease. Yoghurt also aids in immune function and weight control. Based on these health benefits associated with yogurt consumption, there is an increasing demand for yoghurts making it the fastest growing dairy drink product (National Yogurt Association, 2013). The plant raw materials for this study are among the underutilized legumes in Nigeria as well as other African countries. None of them has assumed the status of staple food as maize, rice wheat or cassava. So, the production of yoghurts using these underutilized legumes is a step in the right direction.

2. Materials And Methods

2.1. Collection of Materials

The cereal: millet, as well as the legumes: Bambara nut, Black bean, Pigeon peas, Tigernut and Soyabean were bought from Akwata market, in Enugu State and were identified properly by a plant specialist late Prof. C.I. Ogbonna of the Department of Plant Science and Biotechnology, Abia State University, Uturu. The commercial starter culture Pascaul Greek (Estilo Griego), and 900g Dano tin powder milk were purchased from Shoprite outlet, Abia Mall, Umahia Abia State Nigeria.

2.2. Sample Preparation

The seeds were sorted separately, de-hulled and foreign materials removed especially, unhealthy nuts and seeds which could affect the taste and quality of the yoghurt. 400 g of each of the legumes seeds were measured out, washed and rinsed with potable water, wet milled separately into slurry with 1.5 liters of potable water using cleaned Silver Crest blender model: SC 1589(5000 W), and the milk extracted subsequently from the resulting slurry of each plant by pressured squeeze using muslin cloth. The extracted milk from the separate grain was pasteurized separately to 82 °C between 10 to 15 minutes each and cooled to a temperature of 42 °C. 300 ml of each was transferred to different labeled containers and starter culture introduced.

2.3. Production of Yoghurt Using the Commercial Dano Milk Powder

300 g of commercial Dano milk powder was dissolved in 1liter (1000 ml) of warm water and stirred thoroughly to give a homogenous mixture. The mixture was heated to 82 °C for 10 minutes for pasteurization, and was made to cool to a temperature of 42 °C. 300 ml each was transferred to labeled sample containers, followed by the introduction of starter culture which promoted the fermentation process.

2.4. Formulation of Yoghurts Produced From Five Local Materials and Conventional Milk

Below is the content of the prepared twelve yoghurt samples and the commercial Hollandia yoghurt in alphabetical representation. Sample A, B, C, D, E and F are Dano milk, Soyabean, Tigernut, Black bean and Pigeon pea respectively and fermented with commercial starter culture, while Sample G, H, I, J, K and L are also Dano milk, Soyabean, Tigernut, Black bean and Pigeon pea respectively but fermented with Millet steep water/culture. While Sample M is a plain commercial Hollandia yoghurt that served as a standard for comparison.

Dano milk…………Sample A
Soyabean…………..Sample B
Tigernut……………Sample C
Bambara nut……….Sample D
Black bean...………Sample E
Pigeon pea …………Sample F

Fermented with conventional starter culture

Dano milk…………Sample G
Soybean……………Sample H
Tigernut……………Sample I
Bambara nut……….Sample J
Black bean......……Sample K
Pigeon pea…………Sample L

Fermented with conventional starter culture

Hollandia Yoghurt…Sample M (as a standard control sample) Total of twelve samples of yoghurts were produced.

3. Proximate Analysis

3.1. Determination of Nitrogen/Crude Protein

The micro-Kjedahl method as described in Pearson (1976) was used for the determination of nitrogen/crude protein in the samples. It involved the estimation of the total nitrogen in the sample and the conversion of the nitrogen to protein with the assumption that all the protein in the sample is present as nitrogen. A conversion factor of 6.25, the actual percentage of protein in the samples was calculated as: % crude protein % Nitrogen x 6.25. **Determination of Moisture, Ash, Content,** and **Crude fibre** were determined according to A.O A C Method (1990). **Fat** was determined according to Pearson (1976) method. **Carbohydrate content** was determined according to the method described by A 0 A C (2005)

3.2. Phytochemical Analysis

Determination of **Flavonoid** was according to the method of Boham and Kocipai (1974). **Alkaloids** was done according to Harborne (1973) while other phytochemicals such as **tannins** and **oxalate** were by the method of Pearson (1976) and Saponins was determined according to the method described by Obadoni and Ochuko (2001)

3.3. Physicochemical Analysis

Determination of **Titratable acidity (TA),** was by the method described by AOAC (2010). 5 ml of the sample solution was taken and titrated with 0.1N NaOH using phenolphthalein as indicator. Titration continued until there was a change in colour to a pink endpoint.

$$\text{Titratable acid (\%)} = \frac{T \times M \times 0.09 \times 100}{V}$$

Total Soluble Solids (T.S.S)

The percentage of T.S.S was calculated as shown below:

$$\text{T.S.S. (\%)} = \frac{\quad \times 100}{g}$$

$$\frac{\text{Weight of dry filtrate}}{\text{Volume of sample}} \times 100/1$$

Determination of Milk Solids Non-Fat (M.S.N.F) was done by calculation after the determination of the lactometer reading. %M.S.N. F = 0.25LR + 0.2F + 0.4 % Fat and LR Lactometer reading. **PH** was determined using a Jenway pH meter model 3510

4. Mineral Analysis

4.1. Determination of Phosphorous (Eschka Method)

Phosphorous content was determined by ashing the sample in the presence of zinc oxide followed by colorimetric measurement of phosphorous as molybdenum blue as described by A.O.A C (2015).

4.2. Determination of Sulphur (Eschka Method)

1 g of the pulverized sample was mixed with 3 g of a mixture of magnesium oxide and anhydrous sodium carbonate (2:1). The mixture was heated to 400 ^{0}C for two hours in a muffle furnace. Cooled and digested in water. Barium chloride was added to precipitate the sulphate as barium sulphate. The amount of Sulphur determined as described by (ASTM 1992) and as described by A.O.A C (2015). Sulphur Content (%) =

$$\frac{\text{Ppt } (BaSO_4) \times 0.1373 \times 100}{\text{Weight of Sample}}$$

4.3. Determination of Metals: Calcium, Potassium and Magnesium Content

30 cm^3 of aqua regia (a mixture of HNO_3 and HCI in the ratio of 1:3); de-ionized water, double distilled water, conc. HCI, 3 M HNO_3. Atomic Adsorption Spectrometer model AA-7000 Shimadzu, Japan ROM version 1.01, S/N A30664700709 was used for the analysis of Calcium, Potassium, and Magnesium content.

5. Vitamins Analysis

For determination of **Vitamin A, t**he procedure of Jakutowicz et al (1977) was used. One gram of the sample was weighed. Then, the proteins were first precipitated with 3 ml of absolute ethanol before the extraction of Vitamin A with 5 ml of heptane. The test tube containing this was shaken vigorously for 5 minutes on standing; 3 ml from the heptane layer was taken up in a cuvette and read at 450 nm against a blank of heptane. The standard was prepared and read at 450 nm wavelength and vitamin A calculated from the standard.

5.1. Determination of Thiamin (Vitamin B1)

Complex was extracted with dilute HCl and the resultant solution treated with phosphatase enzyme to liberate free thiamine. 1 g of the sample was weighed into a flask and l00 ml of O.2 NHCI was added and heated to boil for 30 minutes on a water bath. Cooled, 5 ml of phosphatase enzyme added and incubated at 37 °C, filtered and added 2 g of anhydrous Na_2SO4.

5ml of the solution was measured into 5 ml stopped flask and added 3m1 of 15 % NaOH. The absorbance was taken at 435nm wavelength. Thiamin was calculated as:

$$\text{Thiamine} = \frac{\text{Abs of sample}}{\text{Abs of STD}} \times \frac{\text{Conc of STD}}{\text{weight used}}$$

5.2. Determination of Riboflavin (Vitamin B2)

Riboflavin was extracted with dilute acids and then quantified after removing the interfering substances by treatment with $KMnO_4$. 5mg weight of the sample was taken. 50 ml of 0.2 NHCl was added and boiled on a water bath for 1 hour, cooled and the pH adjusted to 6.0 using NaOH. 1 NHCl was added to lower the pH to 4.5 then filtered in a l00 ml measuring flask and the volume was made up to the mark. l0 ml aliquot was taken from l00 ml volume and 1 ml of acetic acid (glacial) was added to each tube, mixed and then 0.5 ml of 3 % $KMnO_4$ solution was added.

Kept away for 2 minutes and then added 0.5m1 of 3 % H_2O_2 and mix well before taking your reading at 470 nm.

Calculate for riboflavin as: $\text{Riboflavin} = \frac{\text{Abs of sample}}{\text{Abs of STD}} \times \frac{\text{Cone of STD}}{\text{weight used}}$

5.3. Determination of Ascorbic acid (Vitamin C)

5 g of sample was weighed into an l00 ml volumetric flasks and 2.5 ml of 20 % meta-phosphoric acid was added as stabilizing agent and diluted to with distilled water. 10ml of the solution was collected with a pipette into a small flask and 2.5 ml acetone added. Titrated with the indophenol's solution until faint pink colour persisted for 15 seconds. Vitamin C was calculated in the sample as mg/100 ml in deeply coloured solution. UV spectrophotometer at wavelength of 264 nm was used in the calculation.

5.4. Analysis of Vitamin E

1 g of sample was weighed into l00mI flask fitted with reflux condenser. 10 ml absolute alcohol and 20 ml M alcoholic sulphuric acid was added. Refluxed for 45 minutes and cooled. 50 ml of distilled water were added and transferred into a separating funnel with acid of further 50 ml of water. Extracted with 30 ml of diethyl ether. The extract was evaporated with very low heat. The residue was dissolved with 10 ml absolute ethanol. Aliquots of the solution and standards (0.3-3.0 mg of Vitamin E) transferred into a 200 ml volumetric flask. 5ml absolute alcohol added, followed by 1ml conc. Nitric acid drop wise with swirling. Placed in a water bath at 90 °C for 3 minutes. Cooled under running tap and adjusted to volume with alcohol. The absorbance was measured at 470 nm against a blank containing 5 ml absolute alcohol and 1 ml nitric acid.

6. Microbial Analyses

The microorganisms in samples were cultivated and identified using surface viable count method

According to the method of Miles and Misra, 1938. *Total Viable Count* **(**number of Living Micro-Organisms). The method used was surface viable count. The suspension obtained from the isolation of bacteria was diluted with sterile distilled water using sterile pipette. The aim was to obtained a dilution that contained approximately 30 cells per 0.01 5 m1 or 0.015 volumes per drop. Agar plates were divided into eight segments with an indelible marker. A drop of the suspension was inoculated on each segment. These plates were then incubated for 24 hours at 37 °C. Developed colonies were counted from the equation as follow:

$$\text{Mean count} — \frac{\text{number of colonies in each segment}}{8}$$

$$\text{Total viable count} - \frac{\text{mean count x dilution factor}}{\text{Vol. per drop}}$$

Dilution factor = 10^4

Volume per drop 0.01 5 ml

6.1. Isolation of Bacteria

One gram of the sample was weighed and transferred into sterile test tubes. Sterile saline solution (10 ml) was transferred to the test tubes containing the samples. The mixture was shaken to obtained uniformity. It was then allowed to set and the supernatant served as the inoculums. Using a sterile loop, a loop full of the supernatant was collected and streaked on the nutrient agar plate. The plates were incubated at 37 °C for 48 hours. After the incubation period, the plates were carefully inspected for growth of bacteria.

6.2. Identification of Pathogenic Bacteria

Some suspected colonies of pathogenic bacteria from the isolation above were identified with Isolation and Identification of Fungi. Selective media Gram-negative rods were grown on Mackonkey agar, cetrimide and desoxcollate citrate agar. Cocci shaped organisms were grown on mannitol agar. The same procedure adopted for isolation and identification of bacteria above was also used for that of fungi in the samples. But in place of nutrient agar, Saboround dextrose agar (SDA) was used. 1 g of the sample was collected with the sterile loop and streaked on SDA plates. The plates were incubated at 25 to 28 °C for 48 hours. The fungi present in each of the samples was identified by microscopy.

6.3. Sensory Analysis

Samples were subjected to sensory evaluation using 9-point hedonic method (9 = excellent; 8= like very much; 7=like moderately; 6=like slightly; 5=neither like or dislike; 4= dislike slightly; 3= dislike moderately; 2= dislike very much 1 = extremely poor). 12 formulated samples the commercial hollandia yoghurt were examined on the basis of their quality attributes such as Aroma, Appearance, Taste, Texture, and Overall acceptability by 36 untrained panelists who were students of JUPEB foundation, ABSU, were recruited and informed about the sensory test.

An informed consent was obtained for sensory experimentation with the panelists and research has been carried out in accordance to Sanful method, (2009).

7. Result And Discussion

The **proximate parameters** measured were ash content, crude fat, crude fiber, crude protein, moisture content, and carbohydrate (Figure 1). 12 yoghurt samples: A B, C, D, E, F, G, H, I, J, K, L (dano milk, soybean, tigernut, bambara, akidi and fiofio yoghurts treated with both commercial culture and millet steep respectively) were compared with sample M- which is a commercial (Hollandia) yoghurt as a control. The **Ash composition of** the products ranged between 0.09 and 1.08% against the control 0.78% with no significant difference among the produced samples as expected ($p > 0.05$). Although some of the samples (soybean, tigernut, akidi and fiofio) recorded lower ash content values, than the value obtained from the control sample M (0.78%), however, it corresponds with the ash content values obtained by other researchers such as (Ihemeje *et al*, 2015 and Joel *et al*, 2014). Also, a similar occurrence was reported 2020 by Nath *et al*, who confirmed lower ash content in Almond and dark chocolate containing yogurt than the control yogurt. The ash in the samples is an indication of the minerals which promote bone formation and mineralization according to FOX report in 1998. A serious significant difference was seen among samples ($p > 0.05$) for **Crude Fat** composition as seen in figure 1 above, it ranged between 1 and 5.85% against 1.84% for the control. All the samples except (akidi and fiofio) recorded appreciable values even above the minimal of NIS337:2004 range of 3.0%. The decrease in fat content recorded in akidi and fiofio samples (between 1.2 and 1.3% on average) may contribute to increased shelf life by decreasing the chances of rancidity, as higher fat content may easily contribute to the production of off flavor during storage (Olakunle, 2012). However, the percentage is in line with minimal daily requirement as recommended by the Dietary Guidelines that recommends 1.5–2% tablespoons for adult women and 2–2.5% tablespoons of oils each day for adult men (ODPHP, 2016). It should be noted that crude fat content of the yoghurts varies with the type of milk and nature of culture used. Yoghurts rich in oil content have been observed to contain “abundant fat and this could be due to the presence of poly-unsaturated fatty acids, which are considered healthy for human body (Ogundele *et al*, 2015). A similar pattern was also observed for the **Crude Protein** content with samples having the range of 1.75 and 5.95% against 2.62% for control (sample M), also showing serious significant difference among samples ($p > 0.05$) as designed. The mean proportional level of protein present in all the yoghurt samples are nutritionally significant in terms of the potentials of these yoghurts to contribute to the increased protein intake by the consumers. The Crude protein has been reported to have some functional attributes such as water absorption, viscosity elasticity, foam stability and fibre formation (Sanful R.E, 2010). The **crude fibre** percentage shows no significant difference (p>0.05) in all the yoghurts samples at the range of 0.1 to 0.5%, in agreement with the control yoghurt sample M (0.2%). A similar result has also been reported by Adriana *et al*, (2018); Raju and Pal (2014). The indigestible components of plant material which include cellulose, hemicellulose, pectin, lignin and other plant material are collectively referred to as crude fibre or dietary fibre. It provides roughages, which contributes to a healthy condition of the intestine (Odom *et al*, 2013). Dietary fibres reduce the risk of cardiovascular diseases caused high blood cholesterol level by decreasing cholesterol level in the body (Anderson *et al*, 2009).

The results showed no significant difference among the samples (p > 0.05) for **Moisture composition**. The values ranged between 78.70 to 94.13% against the moisture content for control sample M (85.92%).

Intriguingly, yoghurts made from akidi and fiofio, recorded the highest moisture content (91.42 and 94.13%). The slightly increments of moisture content observed, could be as a result of reconstitution of the milk prior to fermentation (Ihemeje *et al*, 2015). This is in line with the work of Udeozor (2012) who demonstrated the proximate composition and sensory qualities of tiger nut-soy milk drink, while the moisture contents of some the yoghurts disagree with the range of most commercial yoghurts (80-86 %) as reported by Joel *et al*, (2014). However, moisture can be controlled by the addition of powdered milk or evaporation during pasteurization of milk for desired yoghourt (Stringer, 2000). Another serious significant difference was seen among the samples (p > 0.05) for **carbohydrate composition,** with the least value of carbohydrate obtained in soybean sample B (0.91%) and highest recorded values obtained in (dano milk and tigernut) sample A and C (7.92 and 7.72% respectively) as against 8.64% for sample M, the control. The proximate composition of this analysis is similar to those reported by other researchers (Udeozor; 2012) (Iman, Ogundele, Alhasan and Akoma *et al;* 2013, 2015, 2015 and 2013) respectively.

Proximate composition is very useful for compilers of food composition tables and databases that could be used by economist, food service managers, agricultural planers, nutritionist, dieticians, food and agricultural scientist, food technologist, public health scientist etc. (Greenfield and Southgate, 2003).

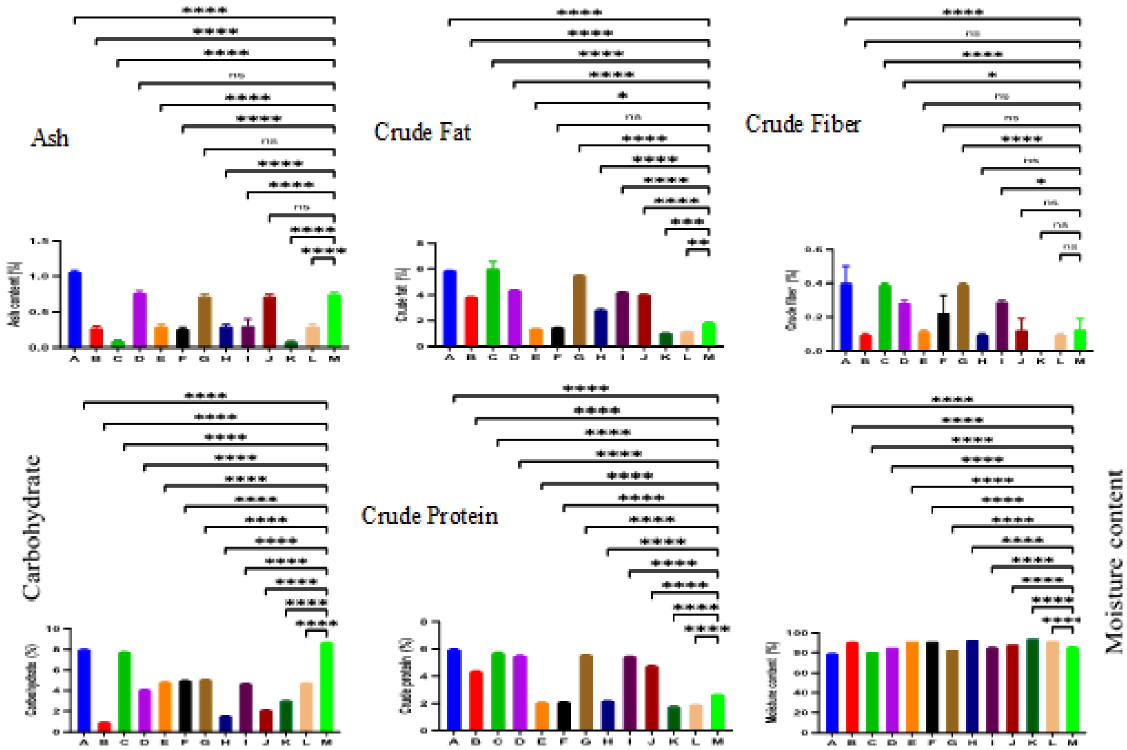

Figure 1. *Proximate configuration of samples fermented with commercial starter and millet steep water*

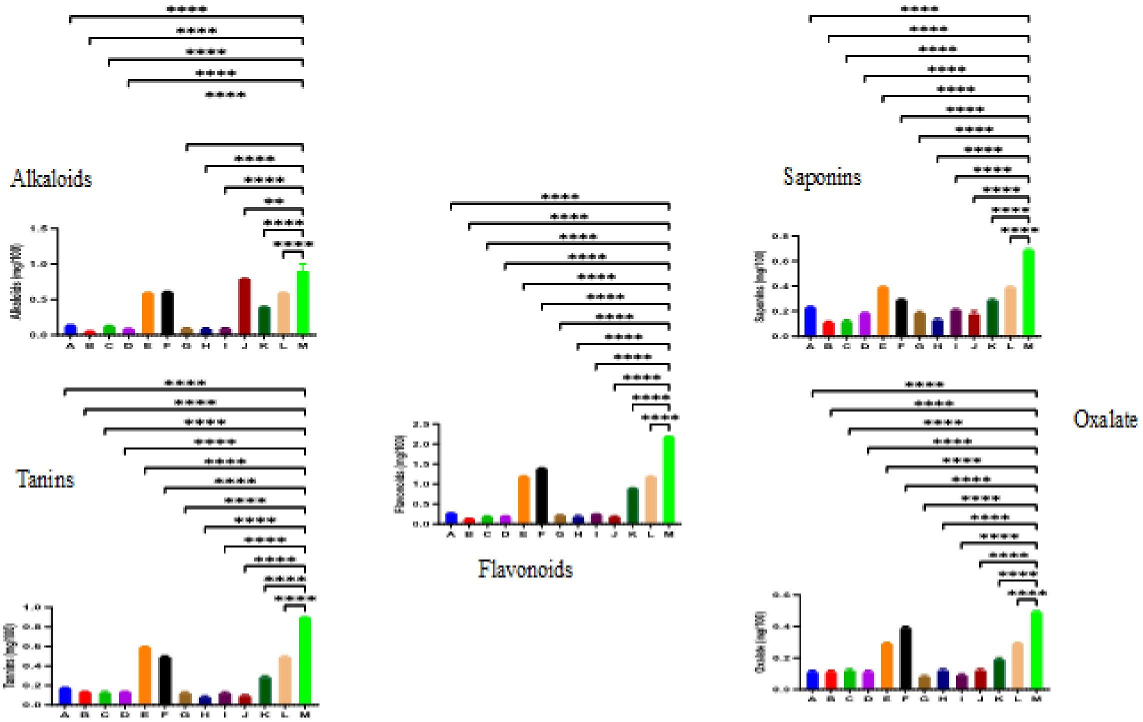

Figure 2. *Phytochemical composition of samples fermented with commercial starter and millet steep water*

The **phytochemical parameters** considered were alkaloids, saponins, flavonoids, tannins and oxalates according to Figure 2 above. The result showed the quantity of alkaloids, flavonoids saponin, tannins and oxalate present in the various yoghurts produced. The quantity of **alkaloid** was significantly lowered (p>0.05) in the yoghurt samples, except in yoghurt samples made of akidi (E) and fiofio (F) fermented with both commercial culture and millet steep (0.6mg) respectively against the control sample (M) 1.0mg. Remarkably, yoghurt made of okpa and fermented with millet (sample J) recorded non-significant increase (p>0.05) with highest concentration value (0.8mg). **Flavonoid** decreased significantly (p>0.05) across the groups. However, samples made of akidi and fiofio (EFKL) recorded higher concentration values of 1.2, 1.4, 0.9 and 1.2mg respectively, while the control sample (M) has 2.2 mg. **Saponin** showed serious significant differences (p>0.05) across the produced yoghurt samples with the least value 0.11mg compared to 0.7mg of the control.

The presence of saponin in moderate concentration is consistent with the report in the literature (Obidoa *eta al*, 2010). Saponin have been shown to reduce blood glucose and insulin responses to starchy foods and or the plasma cholesterol and triglycerides. Also, saponin have been reported to reduce cancer risk (Thompson, 1993). The presence of saponin in the samples could imply that consumption of these yoghurt samples has the potential to lower cholesterol levels in humans due to the hypocholesterolemia effect of saponin (Osagie, 1998). A similar pattern of decrease was also observed for the **Tannin** and **Oxalate** content, with samples having the range between 0.1mg - 0.6mg for tannin, and 0.1mg - 0.3mg for oxalate, and the control sample (M) recorded 0.9mg for tannin; 0.5mg for oxalate, both of them showing serious significant (p > 0.05) differences among the yoghurt samples. It was suggested that the consumption of tannin-containing beverages can cure or prevent a variety of illnesses (Serafini *eta al*, 1994). Also, many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been attributed to tannins (Ifesan *eta al*, 2014). Phytochemicals are important biochemical drivers. Over the years, its wide acceptance has been attributed to the following criteria: bio accumulation, bio availability, higher safety margin and ability to target biochemical pathways (Okereke *et al*, 2017).

Physicochemical properties analyzed include: pH, milk solid non-fat (M.N.S. F) titratable acidity (T. A), total soluble solids (T.S. S), and viscosity (cP) as shown in Figure 3. The result showed varied **pH** levels among the tested samples, ranged between 4.1 minimum to 7.7 maximum for both samples fermented with conventional starter and millet steep. In order word, a serious significant decrease(p<0.05) were recorded among the prepared yoghurt samples A, B, C, D, G, H, I, J. (4.5,4.5,4.1, 4.4, 4.7,4.5,4.5 and 4.5% respectively) except for samples composed of akidi, and fiofio yoghurts sample E, F, K, L. (6.3, 6.4, 7.7 and 6.8% in that order) which recorded little or no significant increase(p<0.05) compared to the control yoghurt sample M (6.4%). Regardless of the decrease in pH of most the yoghurt samples, the various value percentages are optimal for consumption, as the general consensus for the pH value for acceptable and good quality product ranges between 3.5 and 4.6% according to Tugba, 2022; Biberoglu and Ceylan, 2013; Ezeonu, Tatah, Nwokwu, and Jackson, 2016; Tamime and Deeth, 1980; Tomovska, Gjorgievski, and Makarijoski, 2016. Also, 3.38minimun and 4.80maximun for Egyptian Yoghurt Standards (EOSQC), (2005). **Milk solid non-fat** (**MNSF)** composition was non-significantly (p>0.05) lowered in all of the yoghurts regardless of fermented culture ranges between 6.1 minimal to 11.4% maximum as against the control yoghurt M (12.24%). **Titratable acidity (T. A)** of the result showed high significant increase (p<0.05) among yoghurts A, B, C, D, G, H, I, J, (1.49,1.48,1.52,1.51,0.78,1.48,1.44 and 1.49% respectively) except for sample E, F, K, L. (0.1,0.1,0 and 0.05% accordingly) which showed non-significant(p<0.05) difference with the control sample M (0.1%). The titratable acidity of normal fermented milk products ranges between 0.7 - 1.2% (Dello *et al*, 2004 and Ma C *et al*, 2015). However, a study by Choi *et al*, 2016, also recorded titrated acidity ranges between 1.02 - 1.14% after 8hours of fermentation, and was considered normal. The result further showed that there was no statistical difference (p<0.05) in the level of **total soluble solid (TSS)** among the yoghurt samples, except for sample A and H. (12 and 36%) as against the control sample M. (9.86%). The total soluble solids are an indication of the dry matter content of the yoghurt samples (Joel *et al*, 2014; Belewu *et al.*, 2010; Khalifa *et al* 2011). Also, the result of Viscosity was also significantly (p>0.05) lowered in all of the yoghurts samples. The samples of E, F, K, L (94.2, 103.1, 101.6 and 107.1% respectively) competed favorably with the control sample M (164.3%). Excitingly, viscosity results are in agreement with those obtained by Adriana *et al*, (2018); Crispín-Isidro *et al*. (2015) which reported that gel firmness increases at a level of 2–4% inulin addition.

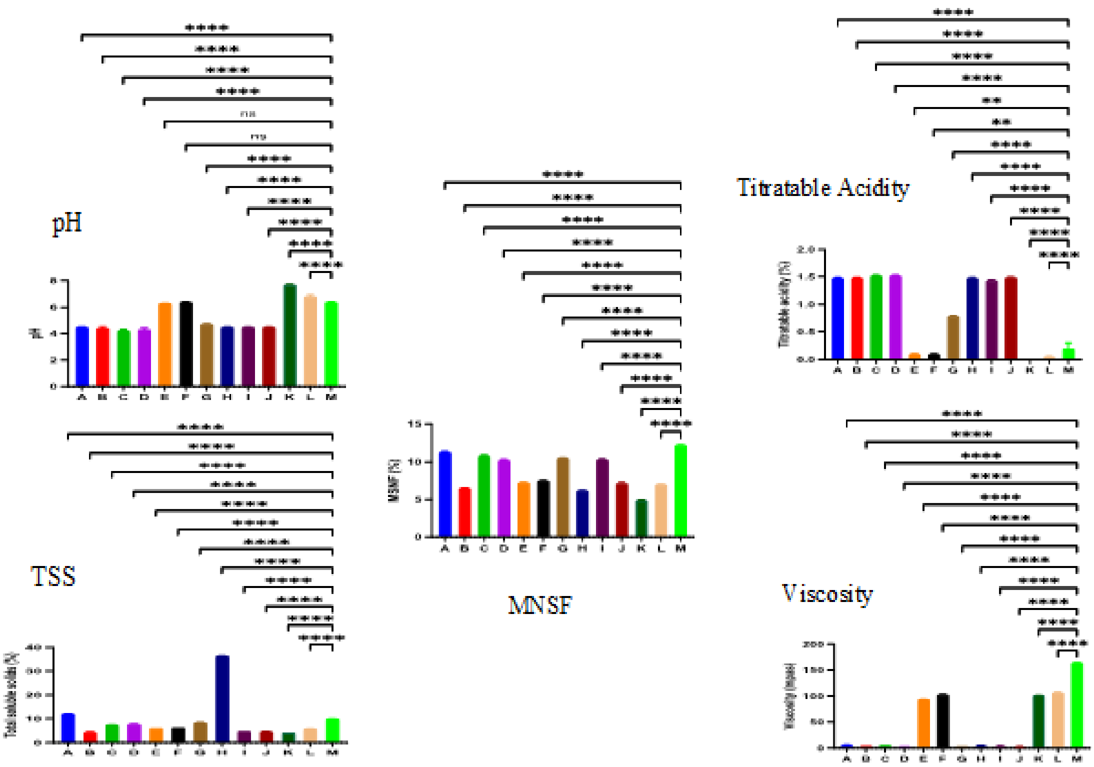

Figure 3. *Physicochemical properties of samples fermented with commercial starter and millet steep water*

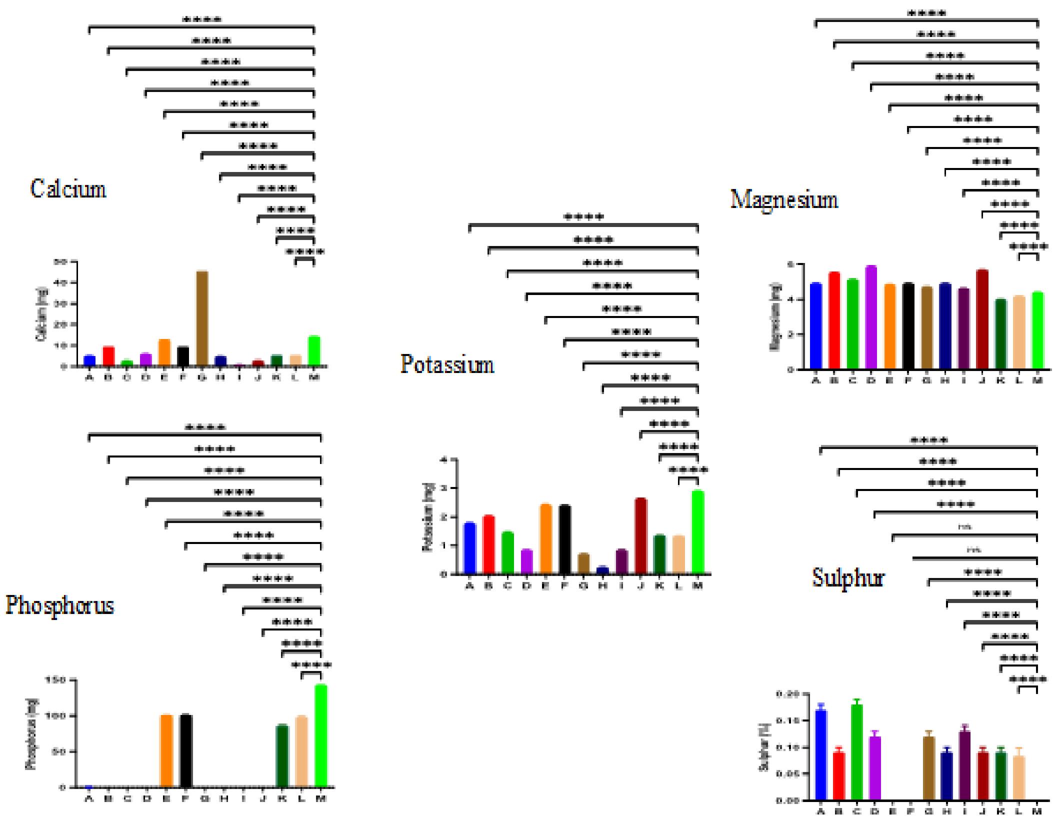

Figure 4. *Mineral content of samples fermented with commercial starter and millet steep water*

The **mineral parameters** evaluated were calcium, potassium, magnesium, phosphorus, and Sulphur contents Figure 4. The results showed that the **calcium** content for all the treatment groups decreased significantly (p<0.0001) except for sample G (45.33%) that showed higher concentration of calcium even greater than the control sample M (14%). The potassium concentration ranged between 0.7 to 2.63% as against the control sample M (2.86%). However, the result showed a significant (p>0.0001) decrease compare to the control yoghurt sample M (2.86%). **Magnesium** significantly (p>0.0001) increased compared to the control sample M. The range was between 3.98minumun - 5.88%maximun against 4.38% recorded by the control sample M. **Phosphorus** content for both treatments (commercial starter culture and millet steep) decreased significantly (p>0.0001) in the experimental groups A, B, C, D, G, H, I, J. (0.2, 0.1, 0.2, 0.2, 0.2, 0.1, 0.2 and 0.1% respectively) except for sample E, F, K, L. (100.2,100.8, 86.2 and 98.4%) compared to the control sample M (142.0%). The result actually means that akidi and fiofio plants are good source of phosphorus.

Sulphur content of all the treatment groups slightly increased non-significantly (p>0.0001) for all the experimental samples. The concentration ranged between 0.0 – 0.2% compared to the control (0.0%). The result of mineral concentration justifies the assertion of Gray (2007) that yoghurt is a very good source of essential minerals needed for human metabolism or functionality of cells (Ihemeje *et al*, 2015). The results also, are in conformity to the work of Mbaeyi *et al.*, (2009) who demonstrated the effect of fermentation on the mineral composition of Ogi (fermented maize) blended with bambara groundnut. However, the results are not in agreement with FDA, (2009) range for (Ca: 132ppm, P: 38.5ppm and Mg: 46.1ppm).

The **Vitamin content** assessed include fat soluble vitamins (B1 and B2), water soluble vitamins (A and E) and ascorbic acid as covered in Figure 5 above. The result shows that the concentration of **Vitamin B1,** were no-significant difference (p>0.0001) irrespective of fermented culture in all of the samples. The range was between 0.01% - 1.8% in comparison with the control yoghurt sample M which recorded 0.04% in vitamin B1 concentration. In a similar way, **Vitamin B2** recorded no-significant difference (p>0.0001) in prepared samples, and ranged between 0.05 – 0.32% as against the control yoghurt sample M, that recorded 0.14%. The trend was extensive to **Vitamin E,** which also shared no-significant difference (p≤ 0.0001) with the concentration ranged between 0.0 - 0.86% compared with the control sample M, 0.02%. **Vitamin A,** recorded a significant decrease ($p \leq 0.0001$) in all the samples concentration regardless of fermented culture, the range was between 0.4 minimum to 4.3% maximum in comparison with the control sample M that recorded 7.9 %. In the opposite conduct, **Vitamin C,** showed a significant ($p \leq 0.0001$) increase in the prepared samples, in a concentration range between 0.6 – 6.1% as against 1.3% of the control sample M. Despite the concentration limits of vitamins recorded in the analysis, the work showed the potentials of the used plants to be a source of different vitamins.

Vitamins on the other hand, are important nutritional components required for the normal functioning of the human body (USDA, 2014).

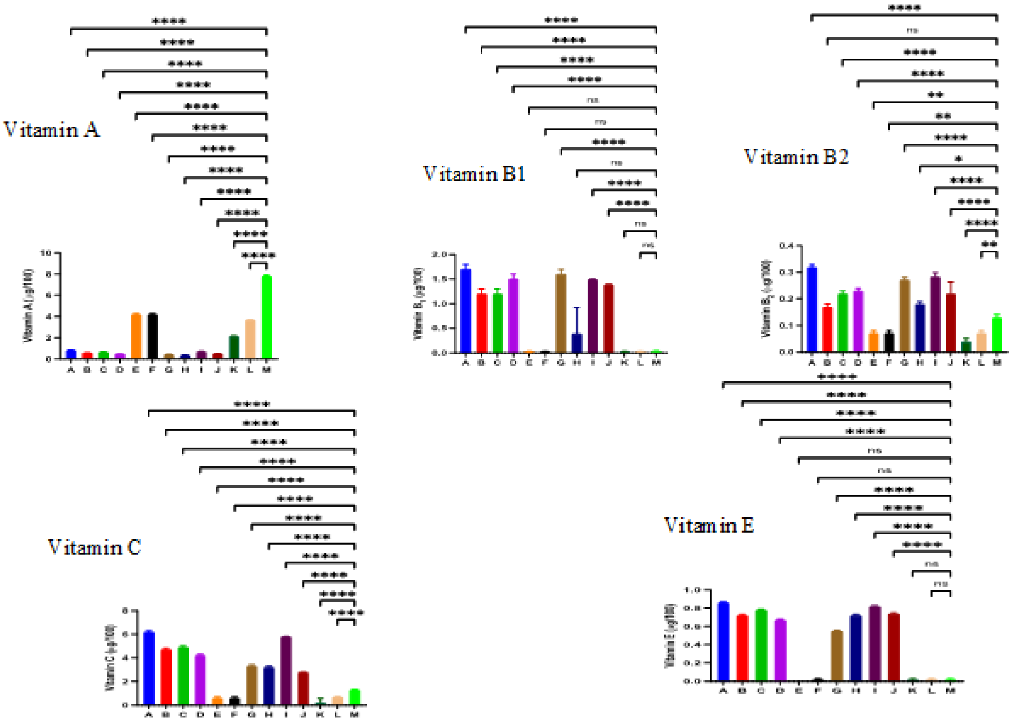

Figure 5. *Vitamin content of samples fermented with commercial starter and millet steep water*

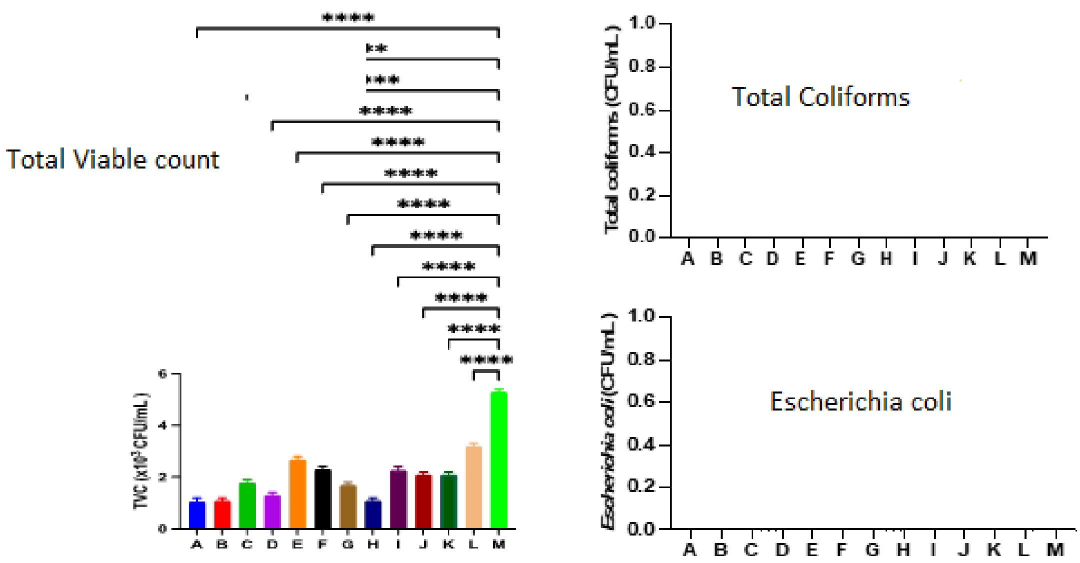

Figure 6. *Microbial analysis of samples fermented with commercial starter and millet steep water*

The studied **microbial include**: *total* viable counts(*TVC*) and potential pathogenic bacteria, such as *total coliform* and *Escherichia coli* as represented in the Figure 6 above. ***Total viable counts*** result showed that both culture treatments were significantly (p<0.0001) decreased for all the yoghurt samples compared to the control sample M.; on the range between 1.0 x 10^3 - 3.3 x 10^3 cfu/ml against 5.4 x 10^3cfu/ml of the control. The analysis further revealed that the total viable counts (*TVC*) of the microbiological analysis reported, is in conformity with the study of Farinde *et al*., (2009) which reported that the standard yoghurt bacterial load range should be < (1x10^6cfu/g). ***Total coliform*** and ***Escherichia coli*, (Pathogenic bacteria)** *were* absent in all the yoghurt samples, suggesting that the yoghurts were safe and suitable for consumption (NIS337:2004). Abrar, *et al* (2009), had reported a similar result. Interestingly, all the yoghurts recorded values within the normal range (6.33cfu/ml and 10.33cfu/ml) (NIS337, 2004) of Nigeria Industrial Standard, (2004), Egyptian yoghurt Standards (EOSQC), (2005) Turkish Standard Institute (1989) and National Yoghurt Association, (2006) all stated that a maximum count of 10cfu/ml of coliform group bacterial is acceptable in yoghurt.

Hence, in this study, the samples with the values less than or equal to 10cfu/ml are therefore justified suitable and safe for consumption. Absence of *Escherichia coli* and *coliforms* as reported will extend the shelf-life of the products.

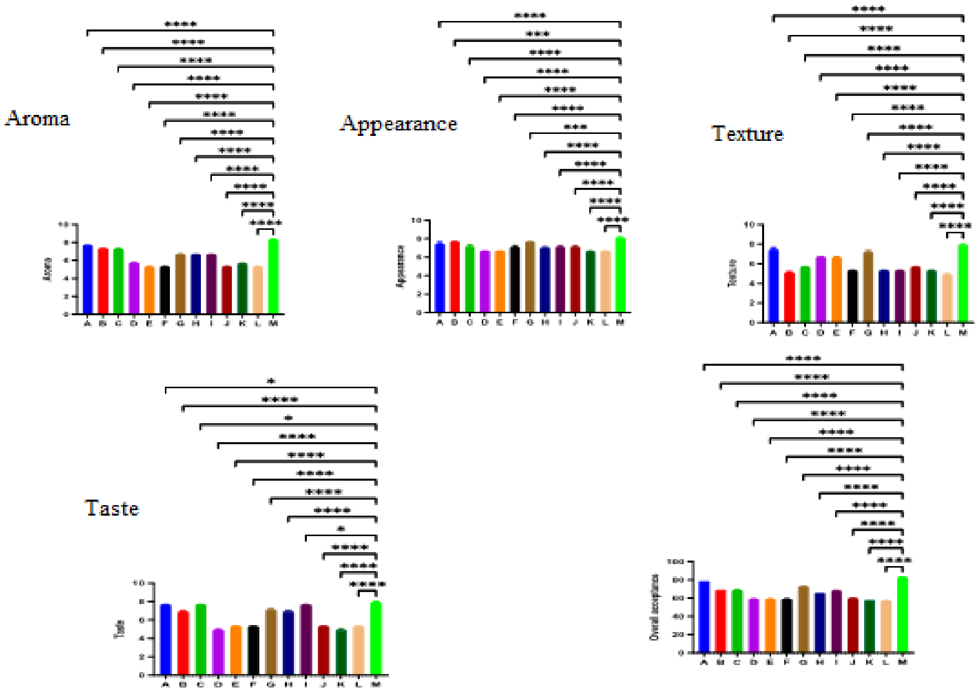

Figure 7. *Sensory analysis of samples fermented with commercial starter and millet steep water*

Aroma, appearance, taste, texture, and overall acceptance were the sensory qualities evaluated as seen in Figure 7. The statistical analysis revealed that there were significant differences (p<0.05) among the yoghurt samples in the sensory attributes observed. The scores ranged between (5.33 - 6.67%) compared to 8.33% recorded by the control sample M. in **Aroma;** 5.0 – 7.67% in **Texture** against 8.0% of the control; and 5.0 - 7.67% compared to 8.0% of the control in **Taste.** There was a slight shift in **Appearance,** as it recorded no significant (p<0.05) increase. The data range was between 6.67 – 7.0% as against the control sample of 8.0%. However, in **overall acceptance,** the results showed more preference of sample A, B, C, G, H and I (78.67, 69.23, 69.23, 72.67, 65.79 and 68.38% respectively) (dano milk, soybean and tigernut) to sample D, E, F, J, K and L (58.97, 58.94, 58.94, 59. 82, 58.13 and 57.27% respectively) (okpa, akidi and fiofio), as it recorded between 57.27% minimal - 78.67% maximum compared to the control sample M (82.9%). The overall results showed that the sensory evaluation response of participants with regard to all the yoghurts were absolutely in relation to the fortification of animal sourced milk (dano) and addition of additives to the control yoghurt M (Hollandia plain yoghurt). The five parameters evaluated were observed to be significantly increased or equal to and to an extent non-significant differ in both the yoghurts fermented with the regular starter culture and millet steep water with appreciable values been recorded across all the parameters evaluated. Interestingly, a similar analysis has been reported by M. Nath *et al*, 2020, and also by Ryan *et al*, 2020 where mango enriched yoghurt showed overall improvement in sensory scores.

8. Conclusions

The study has presented 13 yoghurts formulations, fermented with 2 cultures: conventional starter culture, and millet steep as alternative to commercial starter culture for yoghurt fermentation. Both cultures contain *Lactobacillus bugaricus* and *Streptococcus thermophilus*. The obtained results from all the formulations and control yoghurt, demonstrated that the production of yoghurt from plants raw materials and or integration of plant extracts from tigernut, soybean, Bambara groundnut, Okpa, akidi oji and fiofio is feasible and viable. Also, from the study was seen the feasibility of millet steep water having the potential to ferment milk for yoghurt production. However, there is need for further research on eliminating beany flavour and unhealthy aroma associated with grains and cereals used in the yoghurt production, as unwholesome aroma could discourage yoghurt producers from using the local culture, for the fear of their product rejection by the consumers. The mean proportional level of proteins, carbohydrates, phytochemicals, vitamins and minerals present in all the yoghurt samples are nutritionally significant in terms of the potentials of these yoghurts to contribute to the dietary balanced for dairy consumers. The results obtained from mineral and vitamins contents also justifies the assertion that yoghurt is a very good source of essential minerals needed for human metabolism or functionality of cells, and are also important source of vitamins for nutritional components required for the normal functioning of the human body. Furthermore, low data values of *total viable counts* (*TVC*), the absence of *Escherichia coli* and *total coliforms* as reported here is an indication that the produced 12 samples (A, B, C, D, E, F, G, H, I, J, K and L) are safe for consumption, and will extend the shelf-life of the products. However, the result further revealed that the animal sourced yoghurt recorded the highest overall preference base on the sensory evaluation scores compared to the five plant-based yoghurts (soybean, tigernut, akidi, okpa and fiofio). With this satisfactory results obtained from yoghurt produced from local plant raw materials, it is therefore, advised that individuals should welcome, use and promote yoghurts, beverages and other foods made from full or blends of soybean, tigernut, akidi, okpa and fiofio.S

9. Declarations

- **Ethical Approval and Consent to Participate:** Not Applicable
- **Consent for publication:** The submission of the manuscript to your journal is by our permission.
- **Competing interests:** There is no conflict of interest.
- **Funding:** Self-funding (A PhD research work carried out by me; **Ubiji Chioma Ernest**)
- **Availability of data and materials:** Every information and data herein, are true copy from us.

- **Corresponding Author: Ubiji Chioma Ernest (ubijichiomaernest@yahoo.com)**

10. Authors' s Contributions

- **Prof. Sunday Onyekwere Eze (Supervisor I) -** manuscript reviewer and supervised the sample preparations.
- **Prof. Jude Chibuzo Igwe (Supervisor II) -** manuscript reviewer and supervised the sample preparations.
- **Mr Chioma Ernest Ubiji**. – carried out the research, procured raw materials, formulated, prepared and monitored the samples prior to Laboratory analysis, discussed and analysed the data results.

11. Contributors

- **Dr A. C.** Ofomatah (Chief Research Analyst) – provided equipment for the laboratory analysis.
- **Mr Arunsi, Uche Okuu –** helped with the Statistical analysis

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