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Groundnut Milk Fermentation for Yogurt Production: Physicochemical and Microbial Changes

B.A Omogbai[a*] and I.B. Jacob

[1]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

ABSTRACT: The fermentation of groundnut milk for yogurt production was studied. Groundnut milk was prepared from blended groundnut seeds. Four types of yogurts: GMYP, GMY1, GMY5 and GMY10 were prepared from groundnut milk by fermentation with different levels of sucrose; 0, 1, 5 and 10%. The groundnut milk and yogurts were analysed microbiologically and physico-chemically using standard procedures. Freshly processed groundnut milk had a total plate count of 4.8×102 cfu/ml and low mould (2.8×101cfu/ml) count. No yeasts and coliforms were isolated. Microbial isolates in the groundnut milk include: Bacillus subtilis, Staphylococcus aureus, Pseudomonas sp, Serratia marcescens, Aspergillus niger and Aspergillus flavus. Physicochemical analysis of groundnut milk revealed the following: moisture (92.40±0.85 %), protein (2.75±0.02%), fat (3.18±0.05 %), ash (0.51±0.00 %) and carbohydrate (1.16±0.01 %). The total dissolved solids, titratable acidity and pH were 7.62±0.56 %, 0.15±0.00 % and 6.61±0.01 respectively. The physicochemical properties of fermented groundnut milk yogurts showed the percentage moisture, protein, lipid, ash and carbohydrate contents were in the range 92.80±0.61-94.81±0.67, 1.87±0.05-2.37±0.12, 1.25±0.05-1.67±0.02, 0.50±0.00-1.20±0.00 and 0.65±0.02-2.88±0.03 in that order. Yogurts fortified with 5 and 10% sucrose had the lowest pH of 4.30±0.00 and 4.35±0.00 respectively. Acid formation was considerably higher for the fortified yogurt. The final viable cell counts in the yogurts fortified with 5 and 10 % sucrose (GMY5 and GMY10) were 8.73Log10cfu/ml and 8.85Log10cfu/ml respectively. These high counts of bacteria with acid production are considered acceptable for a typical yogurt. Production of groundnut milk yogurt is possible with supplementation with sucrose.

Keywords: Groundnut milk, fermentation, yogurt, chemical content, microbial

Introduction

Groundnut (*Arachis hypogeae)*, originated in Bolivia and adjoining countries but is now grown throughout the tropical and warm temperate regions of the world. Groundnut is a species in the legume or "bean" family (*Fabaceae*). It is an annual herbaceous plant that grows 30cm to 50cm (1.0-1.5ft) tall. The leaves are opposite, pinnate with four leaflets. The pods have outer, thick woody shell with two to three seeds embedded inside (Singh and Diwakar, 1996). The seed is an important food source of proteins and its oil is one of the major oils in the human diet. Groundnut seed contains 40-50% oil, 22-32% protein and considerable amounts of minerals (Yagoub and Ahmed, 2012).

Groundnuts are consumed mostly as processed products. Like soybean, groundnut can be processed into groundnut milk. The groundnut milk which has high protein content is extensively used in India and other developing countries by vegetarians (Pangastuti *et al.*, 2011). The current interest in peanut milk and peanut milk products is motivated by the fact that dairy and dairy products are always priced too high for the low income earners. Another factor, no less important is the growing awareness of the nutritional benefit of vegetable proteins in cholesterol diet by health conscious people. Protein-energy malnutrition is the most common deficiency disease in the world, especially in developing countries. This kind of malnutrition is related mainly to inadequate quantity and low quality of protein food and therefore more protein foods are needed. Accordingly, the world attitude is oriented towards developing low cost protein foods of plant origin, especially for low income groups in developing countries (Yagoub and Ahmed, 2012). Since groundnut has a potential role to play in combating malnutrition, the present low level in its consumption, especially in the developing countries, should be increased. Fermentation of groundnut milk will serve as one such effort that can increase the protein availability and consumption (Razig and Yousif, 2010). Groundnuts may be consumed raw, roasted, pureed or in a variety of other processed forms and constitute as a multi-million dollar crop worldwide (Isanga and Zhang, 2007).

Groundnut is a rich source of energy due to its high oil and protein contents. It supplies about 5.6 calories per grain when consumed raw and 5.8 calories per grain when consumed roasted. It is a rich source of essential amino acids, minerals and vitamins. Groundnut has good digestibility in both raw and roasted forms (Singh and Diwakar, 1996).

Groundnut is composed of protein (25.2%), oil (48.2%), starch (11.5%), soluble sugar (4.5%), crude fibre (2.1%), and moisture (6.0%). It has also been reported to contain essential amino acids such as lysine, threonine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine and tyrosine in various amounts (Singh and Diwakar, 1996). According to Singh and Diwakar, (1996) it was reported that antimicrobial factors such as trypsin and enterokinase inhibitors are found in groundnuts.

Groundnut milk is the water extract of groundnut that is an inexpensive source of proteins and calories for human consumption. Preparation and fermentation of peanut milk may serve as one such effort that can increase the consumption of this valuable crop and hence improve protein availability and consumption (Sunny-Roberts *et al*., 2004). Groundnut milk may be produced by soaking and grinding full fat raw peanuts with water to obtain slurry and subject to filtration (Giyarto *et al*., 2011). Groundnut milk may also be produced by grinding un-soaked roasted groundnuts, raw full-fat or partially defatted groundnuts to form flour to which water may later be added to make an emulsion (Isanga and Zhang, 2007). In many cases the peanut or peanut flour to water ratio vary depending

*Corresponding authors E-mail: barryomogbai@yahoo.com

on the producer. The milk-like product is then homogenised, pasteurised and supplemented with vitamins and minerals with the addition of flavours (Isanga and Zhang, 2007).

Yogurt is a coagulated milk product, which results from the fermentation of milk by probiotic lactic acid bacteria for example, *Lactobacillus bulgaricus* and *Streptococcus thermophillus* (Ershidat *et al*., 2009). The growing popularity of yogurt over the years has largely been increased due to its perceived health benefits. Yogurt is one of the best fermented dairy foods that contain "probiotics" which are living microorganisms; upon ingestion in sufficient amount exert beneficial effects on the normal microbial population of the gastro-intestinal tract (Ershidat *et al*., 2009). Other effects include enhancing the immune system, synthesizing and enhancing the bioavailability of nutrients and reducing symptoms of lactose intolerance (Parvez *et al.,* 2006).

This study aims at investigating the microbial load of groundnut milk, and the suitability of the milk for preparing dairy analog especially for lactose-intolerance population coupled with the fact that pure dairy products are quite expensive. Special thought is therefore given to inoculum development of the common yogurt bacteria, preparation of bland groundnut milk, supplementation with sucrose instead of cow milk and fermentation procedures.

Materials and Methods

Collection of Samples

Groundnut (*Arachis hypogaea*), used were purchased from Uselu market, Benin City, Edo State, Nigeria. The lactic acid bacteria starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were derived from a commercial yogurt. The isolates were identified and characterized using the methods of Buchanan and Gibbons (1974) and Collins *et al.* (2004).

Preparation of Groundnut Milk

The groundnuts were first thoroughly cleaned by picking all the stones and other foreign particles present in them while sorting out the good ones. The cleaned groundnuts were weighed (200g) using an electronic scale (KERRO. Model number: KLS10001) and soaked in cold water for 18h, drained and dehulled manually and immersed in boiling water for 20 minutes (blanching). The seeds were then blended with one litre (1.00 L) of boiling water using a Binatone blender (model number: BLG-450, China). The resulting mixture was sieved using a double layered cheese or muslin cloth to obtain groundnut milk.

Sterilization of Groundnut Milk

The groundnut milk was sterilized in an autoclave for 15 minutes at 121^0C and cooled to about 43^0C prior to inoculation with the microbial cultures for fermentation.

Inoculum Preparation

Pure cultures of isolates were employed as starter cultures in this experiment. The cultures were maintained according to the method of Omogbai *et al.* (2007). The stock cultures which were acclimatized in groundnut milk for 168h was used in inoculum development as described below. A 5ml vial of the pure culture was used to inoculate each first-set flask. These subcultures were incubated at 44^0C for 24h. Second -set flasks were inoculated with 5% by volume inoculum and incubated for 12h at 44^0C. Third -set flasks were inoculated with a 5% by volume inoculum, incubated for 3h at 44^0C and then used as the inoculum for the study. In this experiment all inocula were subcultured as pure cultures up to the third-set flasks. The fourth-set flasks were inoculated with a mixed culture of the two pure isolates (Omogbai, 1994).

Production of Groundnut-Milk Yogurt

Four different fermentation types was carried out in this experiment. The first set of experiment was the fermentation of plain groundnut milk (GMYP). In the other three the groundnut milk were fortified with various concentrations of sucrose as follows 1% (GMY1), 5% (GMY5) and 10% (GMY10) respectively. In this study groundnut milk fermentation was conducted in 500ml conical flask (Pyrex, England). Aseptic conditions were maintained in the flasks by use of cheese cloth-covered cotton plugs and aluminium foils. Five litres of groundnut milk were autoclaved in a 6 L flask fitted with cheese cloth-covered cotton plugs and aluminium foils. A 5% volume of total inoculum consisting of a mix from each pure culture flask was added to a sufficient quantity of sterile groundnut milk in a 6 L Erlenmeyer flask. Pure cultures were mixed in four sterile 250ml inoculum flasks using a mechanical mixer so as to introduce a uniformly mixed culture of bacteria into the groundnut milk. The entire contents of the 6 L flask were mixed for approximately 2min. Sterile 500ml flasks were filled aseptically, placed onto a tray and incubated at 44^0C for fermentation. Sampling was carried out every 1h during the fermentation. One flask was removed at each sampling time and served as the sample for that portion of the experiment. Fermentation lasted for 24h following the modified method of Omogbai (1994).

Physico-chemical Analyses:

The pH value of groundnut milk and groundnut yogurt samples were measured with a digital pH meter (model RL10CTQ, Thermo Rusell Inc, USA). Estimation of titratable acidity (TA) was by acid-base titration procedure of AOAC (2000) in which 10 mL of sample was titrated against 0.1M NaOH using 1% phenolphthalein as an indicator.

Proximate composition/Analyses

Moisture and total solids content of samples (groundnut milk and groundnut yogurt) were determined based on the principle of drying to constant weight (Osborne and Voogt, 1978). Lipid content was measured using the soxhlet extraction method. Crude protein was estimated by determination of the total nitrogen content by kjeldahl method (AOAC, 2000). The ash content of each sample was determined by drying in a muffle furnace at 500^0C (Pearson, 1976). Carbohydrate composition was obtained by difference. Thus carbohydrate content was determined by adding up the fibre, protein, lipid and ash and subtracting it from 100.

% carbohydrate = 100- (% protein+ % lipid+ % fibre+ % ash) (AOAC, 2000).

Microbiological Analyses

Media

Nutrient agar was used for enumeration and isolation of bacteria while potato dextrose was used for isolation of fungi in the groundnut milk. Lactic acid bacteria in groundnut milk yogurt were enumerated with MRS agar. All media used were prepared according to manufacturer's instructions. They were sterilized at 121^0C for 15 minutes. On cooling to about 45^oC, the medium was aseptically poured into sterile Petri dishes and allowed to solidify. Microbial enumeration of the samples was by the pour plate technique. Bacterial cultures were incubated for 24h while those of fungi for 72h (Collins *et al.* 2004).

Isolation of microorganisms

The groundnut milk and yogurt were serially diluted using peptone water. An aliquot 0.1ml of the appropriate dilution of groundnut milk and yogurt was plated in Nutrient, Potato dextrose agar and MRS agar. Representative colonies on the culture plates were successively sub-cultured into fresh agar plates of the same medium until pure cultures were obtained. Bacteria were identified according to the methods of Buchanan and Gibbons (1974) and Collins *et al.* (2004). Fungi were identified based on the methods of Barnett and Hunter (1998).

Results and Discussion

In Table 1 is shown the microbiological characteristics of groundnut milk. The total plate count and mould count were 4.8×10^2cfu/ml and 2.8×10^1 cfu/ml respectively. No yeast and coliforms were isolated from the groundnut milk. Microbial isolates from the milk include *Staphylococcus aureus*, *Bacillus subtilis*, *Serratia marcencens*, *Pseudomonas sp*, *Aspergillus niger* and *A. flavus*. The proximate and physicochemical analysis of groundnut milk processed in the ratio of 1:5 in distilled deionised water is shown in Table 2. The milk was found to contain moisture (92.40±0.85 %), protein (2.75±0.02%), fat (3.18±0.05 %), ash (0.51±0.00 %) and carbohydrate (1.16±0.01 %). The total dissolved solids, titratable acidity and pH value were 7.62±0.56 %, 0.15±0.00 % and 6.61±0.01 respectively. In Table 3 is shown the physicochemical properties of fermented groundnut milk yogurts. The percentage moisture, protein, lipid, ash and carbohydrate contents were in the range 92.80±0.61-94.81±0.67, 1.87±0.05-2.37±0.12, 1.25±0.05-1.67±0.02, 0.50±0.00- 1.20±0.00 and 0.65±0.02-2.88±0.03 in that order. In general, plain groundnut milk yogurt (GMYP) had a higher value of protein (2.37±0.12 %) compared to the yogurts fortified with sucrose. Groundnut milk yogurts fortified with sucrose (GMY1, GMY5 and GMY10) had higher concentrations of carbohydrate, ash and total solids (Table 3) compared to GMYP (plain groundnut milk yogurt). The final titratable acidity of sucrose fortified yogurts were considerably higher (0.62±0.02-0.81±0.01 %) in comparison to unfortified yogurt (GMYP) with 0.53±0.00 %. The final pH of the yogurts was in the range 4.30±0.00- 4.95±0.00. Yogurt fortified with 5 and 10% sucrose had the lowest pH of 4.30±0.00 and 4.35±0.00 respectively.

Table 1: Microbial Counts and Synopsis of Microbial Isolates of Freshly Prepared Groundnut Milk

Parameter	Amount present
Total plate count (cfu/ml)	4.8×10^2
Yeast (cfu/ml)	Nil
Moulds (cfu/ml)	28
Coliforms (cfu/ml)	Nil
Microbial isolates	*Staphylococcus aureus*
	Bacillus subtilis
	Serratia marcencens
	Aspergillus niger
	Aspergillus flavus
	Pseudomonas sp

Table 2: Proximate and Physicochemical Composition of Freshly Prepared Groundnut milk

Parameter	Amount (%)
Moisture	92.40±0.85
Protein	2.75±0.02
Lipid	3.18±0.05
Ash	0.51±0.00
Carbohydrate	1.16±0.01
TDS (Total dissolved solids)	7.±0.56
Titratable acidity	0.15±0.00
pH	6.61±0.01

Table 3: Physicochemical Properties of Groundnut Milk Yogurts

Parameter (%)	GMYP	GMY1	GMY5	GMY10
Moisture	94.81±0.67	93.75±0.52	93.30±0.34	92.80±0.61
Protein	2.37±0.12	2.21±0.15	2.10±0.00	1.87±0.05
Lipid	1.67±0.02	1.56±0.00	1.47±0.03	1.25±0.05
Ash	0.50±0.00	0.98±0.01	1.18±0.00	1.20±0.00
Carbohydrate	0.65±0.02	1.50±0.04	1.95±0.00	2.88±0.03
TDS (Total dissolved solids)	5.19±0.26	6.25±0.30	6.70±0.05	7.00±0.00
Titratable acidity	0.53±0.00	0.62±0.02	0.75±0.01	0.81±0.01
pH	4.95±0.00	4.56±0.00	4.30±0.00	4.35±0.00

In Figs 1 and 2 are shown changes in pH value and titratable acidity of fresh groundnut milk during fermentation. The initial pH (6.61) decreased sharply during the first few hours of fermentation with the inoculated lactic acid bacteria (LAB) and then slightly afterwards. The titratable acidity increased steadily during the 20-hour period of fermentation (Fig 2).

From Fig 3, it can be observed that the viable cell count of inoculated LAB increased from the initial count of 6.20 Log_{10}CFU/ml to 8.58Log_{10}CFU/ml after a 20-hour fermentation of plain ground nut milk (GMYP). The bacteria growth in sucrose fortified yogurts was steady with sharp increase at first which later declined to final values. The bacteria cell concentration in GMY10 was more compared to the rest yogurts. The initial cell concentration increased from 6.20 Log_{10}CFU/ml to 9.50 Log_{10}CFU/ml in 8h and then decreased steadily to a final 8.85 Log_{10}CFU/ml in 20h. GMY5 was at par with GMY10 in LAB cell concentration with an initial cell count of 6.20 Log_{10}CFU/ml which increased to 9.21 Log_{10}CFU/ml in 12h and then finally to 8.73 Log_{10}CFU/ml in 20h.

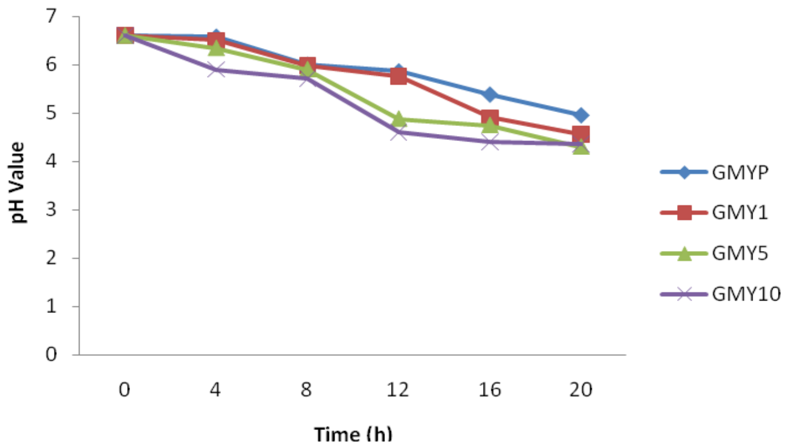

Fig. 1: Changes in pH in Fermenting Groundnut milk Yogurts

GMYP = Plain groundnut milk yogurt
GMY1 = Groundnut milk yogurt fortified with 1% sucrose solution
GMY5 = Groundnut milk yogurt fortified with 5% sucrose solution
GMY10 = Groundnut milk yogurt fortified with 10% sucrose solution

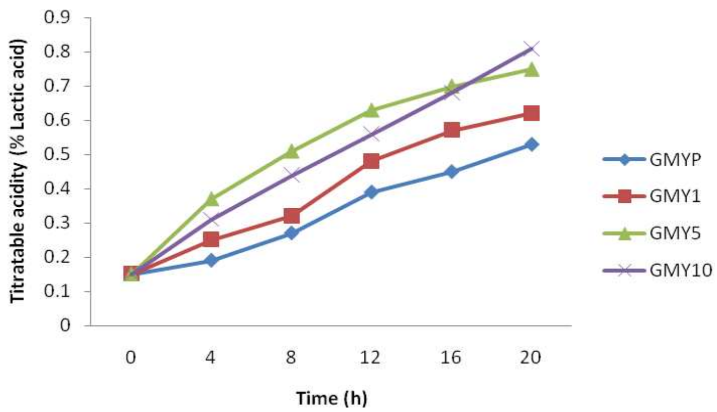

Fig. 2: Changes in Titratable Acidity in Fermenting Groundnut milk Yogurts

GMYP = Plain groundnut milk yogurt
GMY1 = Groundnut milk yogurt fortified with 1% sucrose solution
GMY5 = Groundnut milk yogurt fortified with 5% sucrose solution
GMY10 = Groundnut milk yogurt fortified with 10% sucrose solution

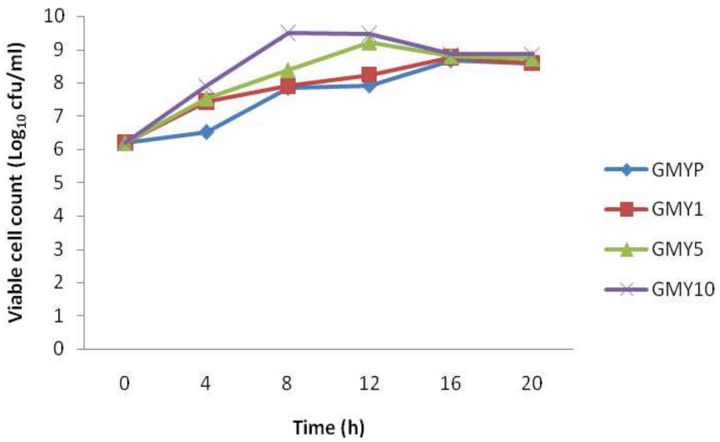

Fig. 3: Changes in Viable Cell Count of Lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) during Fermentation of Groundnut milk Yogurts

GMYP = Plain groundnut milk yogurt
GMY1 = Groundnut milk yogurt fortified with 1% sucrose solution
GMY5 = Groundnut milk yogurt fortified with 5% sucrose solution
GMY10 = Groundnut milk yogurt fortified with 10% sucrose solution

The microbiological quality of fresh groundnut milk samples (Table 1) as determined was within acceptable limits. The total bacterial count and fungal count of 4.80×10^2 and 2.80×10^1 cfu/ml are quite low. The low count obtained could be due to the method of preparation of the groundnut milk. The use of boiling water in the blending of groundnuts, though helps in reducing the n-hexanal content thus reducing the beany flavour of groundnut milk also served to sterilize the milk. The isolates obtained on microbiological analyses were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas sp, Serratia marcescens*, *Aspergillus niger* and *Aspergillus flavus*. The groundnuts must have been contaminated by *B. subtilis* on the farm being one of the major soil microflora which produces spores resistant to the high temperatures of the boiling water used. *Staphylococcus aureus* which is a major food contaminant and a resident flora of the skin must have contaminated the groundnut milk during the filtration process using the double layered cheese cloth. *Pseudomonas sp* and *Serratia marcescens* are also contrbuted from soil (Adams and Moss, 2008). The moulds *Aspergillus niger* and *Aspergillus flavus* are frequently associated with farm produce such as nuts where they not only cause spoilage but produce aflatoxin (Bibek, 2005). The absence of coliforms and yeasts possibly denote good manufacturing practice.

It has been shown that the ratio of groundnut to water in the preparation of groundnut milk affects its chemical composition. In this study, groundnut milk was prepared for production of groundnut milk yogurt using the groundnut to water ratio of 1:5 (200g groundnut blended with 1000ml of water) and the proximate composition is as shown in Table 2. Sunny-Roberts *et al*. (2004) used a ratio of 5:7 and obtained lower moisture content with increased fat, carbohydrate and protein content. The protein level of groundnut milk prepared from groundnut to water ratio of 1:10 and 1:20 were 2.26% and 1.41% respectively (Giyarto *et al*., 2011). This study produced groundnut milk with a higher protein content of 2.75% using a ratio of 1:5. The fat content of 3.18% obtained was also found to be higher compared to that obtained when a ratio of 1:10 and 1:20 was used by other researchers (Razig and Yousif, 2010; Isanga and Zhang, 2009).

The present study shows that fermentation of groundnut milk by lactic acid bacteria (LAB) affects its proximate composition. A Comparison of Tables 2 and 3, reveals that groundnut yogurt had reduced amounts of protein, carbohydrate, fats, ash, dissolved solids, with a higher moisture composition. The lower protein content of the groundnut milk yogurts is indicative of proteolysis during fermentation. Although most starter cultures for yogurt production are weakly proteolytic, Tamime and Robinson (1999) reported that *Lactobacillus bulgaricus and Streptococcus thermophilus* can cause significant degree of proteolytic activity during fermentation. Proteolysis is vital because enzymatic hydrolysis of milk proteins results in the liberation of peptides and free amino acids which in-turn contributes to gel formation and the physical structure of the yogurt formed (Tamime and Robinson 1999). The lower fat or lipid content of the yogurts can be attributed to lipid metabolism by the LAB culture used and homogenisation. Lipolysis in homogenised milk is usual greater than in non-homogenised milk due to the destruction of the protective layer of the fat globule membrane. The low levels of fat in the yogurts may be advantageous to the keeping quality as changes in rancidity will be reduced (Giyarto *et al.,* 2011).

The initial pH and titratable acidity of fresh groundnut milk obtained from this study were 6.61±0.01 and 0.15±0.00%. This is similar to that obtained by Yagoub and Ahmed, (2012). However the pH as expected is higher compared to the fermented yogurts. The titratable acidity was lower compared to the yogurts because as fermentation progressed, acidity increased (Figs 1 and 2). The range of titratable acidity 0.53±0.00 -0.81±0.01 is in agreement with the recommendation of International Dairy Federation (IDF) which suggested a minimum of 0.7% lactic acid for commercial yogurts (Tamime and Robinson 1999). The pH range (4.30±0.00-4.95±0.00) of the yogurts processed in this study is considered acceptable for yogurts (Matalon and Sandine, 1986). A low pH and high titratable acidity ensures the good keeping quality of yogurts.

The microbial count of starter culture inoculated was 6.20 log_{10} CFU/ml. There was a sharp increase in the count during the first few hours of fermentation which became static for a while and then a gradual decrease in the count was observed to the end of the fermentation period. Patel *et al*. (1983) reported that the growth of lactic acid bacteria is vitally important for acid development and proteolysis which in turn enhance good flavour in yogurt. The final viable cell counts in GMYP, GMY1, GMY5 and GMY10 were 8.58Log10cfu/ml, 8.60Log10cfu/ml, 8.73Log10cfu/ml and 8.85Log10cfu/ml respectively (Fig 3). The high counts of bacteria with acid production may be attributed to symbiosis and presence of fermentable substrates.

In conclusion, the results of this investigation confirmed that lactic acid bacteria can grow well and cause the fermentation of groundnut milk into yogurt. Groundnut milk yogurt contains nutrients of fat, protein and carbohydrate which is needed for maintenance of health and the avoidance of malnutrition. Since Protein-energy malnutrition is the most common deficiency disease in the world, especially in developing countries like Nigeria, it is therefore recommended that our attitude be oriented towards developing low cost protein foods of plant origin like groundnut milk yogurt, especially for the low income groups. The groundnut milk yogurt, can also be utilised by individuals allergic to cow milk proteins, hence will be a good dairy analog.

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