Microbiological Quality of a Yoghurt-Like Product from African Yam Bean

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Abstract: Yoghurt was produced from African yam bean and studied. Milk was extracted from whole and dehulled seed African yam bean seeds, pasteurized and fermented with yoghurmet (a commercial yoghurt culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*). Microbial qualities of the yam bean yoghurt samples were evaluated to determine the microbiological quality of the products. Microbial load decreased significantly ($p<0.05$) with increase in storage time. Bacterial species isolated from the yoghurt samples were *Lactobacillus* sp and *Streptococcus* sp. Spoilage by yeast were also noted at room temperature.
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1. INTRODUCTION

Yogurt which is a product of Lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus* is one of the most traditional cultured milk (Steinkraus, 1997). Yoghurt is not just seen as a diet food but also a health food because of its therapeutic value and it is consumedas both as a food and a thirst quenching beverage (Alfa-Lawal, 1984). Increasead yoghurt consumption enhances the immune system due to the presence of yoghurt starter and probiotic bacteria which should be present at recommended concentration of log 6 to 8cfu/g at the time of consumption (Vasiljevic and Shah, 2008)

The African yam bean (AYB) though a minor underexploited legume is an important crop in Western Africa consumed within a geographical region in Nigeria (Njoku *et al.,* 1989). The seeds are delicious and have crude protein level varying from 21% - 29% lower than soyabean (38%) (Evans and Bouther, 1974). It however has lysine and methionine levels equal to or better than those of soyabean (Okigbo, 1973). Since legumes are important sources of relatively inexpensive protein, the introduction of imitation fermented milk products from legumes my contribute to the alleviation of protein malnutrition (Rao *et al.,*1998).

The aim of this study is to determine the microbiological quality of a yogurt-like product from African Yam Bean.

2. MATERIALS AND METHODS

2.1. SOURCE OF AFRICAN YAM BEAN (AYB)

The African yam bean (cream coloured variety) was purchased from Umuahia main market, Abia State. The bean was packaged in polyethylene bags, transported to the laboratory and kept at 4^0C until required. Yogourmet Freeze-dried yogurt starter (Lyo San Inc.,Canada) was gotten from Ariara market in Aba.

2.2. PRODUCTION OF AFRICAN YAM BEAN MILK AND YOGURT

African yam bean seed was divided into two portions and one portion was milled in a sample mill and passed through a 0.5mm screen to obtain flours. The African yam bean milk was prepared from the flour as described by (Aminigo et al, 2007). Each bean flour was blended with 85^0C hot water (1:4 seed : water) in a blender for 3min.The resulting slurry was filtered through two layers of double folded cheese cloth and coarse particles were removed by allowing the filtrate to settle for 10min. The second portion was soaked in 4% NaCl for 12hr, heated for 5min at 100^oC, dehulled and blended with water (1:4) for 3min.The slurry was filtered through two layers of double cheese cloth. Soybean seed was milled and passed through a 0.5mm screen to obtain the flours and the milk was also prepared from the flour as described by (Aminigo *et al*., 2007). African yam bean milk and soybean milk were supplemented with 4% skim milk powder, 1.25% sucrose and 0.5% gelatine. The mixture was heated with stirring to 95^oC for 20 min and cooled to 43^oC. The milk was then inoculated with starter culture (5g in litre milk) and incubated at ambient temperature for 12hr. The yogurt was stored at 4^oC and at room temperature for 21 days.

2.3. MICROBIOLOGICAL ANALYSIS

Microbiological quality of "AYB Yogurt" was evaluated by enumerating total viable organisms at 4^0C and at room temperature. The organisms enumerated include yeast and moulds, total coliforms and *Escherichia coli and Lactobacilli.* These were done according to procedures outlined in the Compendium of Methods for the Microbiological Examination of

Foods (Speck, 1976). The media used in this study were nutrient agar, potato dextrose agar, simmon citrate agar, MacConkey agar, Eosin methylene blue agar, M.R.S agar, tryptone broth and Hugh and Leifson's or oxidation-fermentation (O-F) medium.

2.3.1. DETERMINATION OF MICROBIAL LOAD OF "AYB YOGURT"

Ten grammes of the AYB-Yogurt samples were mixed with 90 ml normal saline (0.85% NaCl) to obtain a 10^{-1} dilution. Further ten-fold serial dilutions were made using the same diluents till a dilution of 10^{-5} was obtained. The microbial population was assessed by the spread plate technique. Aliquot (0.1 ml) of suitable dilution was spread plated in triplicates onto prepared and dried petri-plates of suitable media for the enumeration of different organisms. The total viable count, Lactic acid bacteria, Potato Dextrose agar (PDA Oxoid) acidified to pH 3.5 with lactic acid. Total coliforms and *E. coli* counts were carried out. Serially diluted yogurt samples were also surface plated onto plates of Eosine Methylene Blue agar in duplicate (Oxoid CM 69) and incubated at 44.5°C for 24 hours for the enumeration of *Escherichia coli*.

2.3.2. ISOLATION AND IDENTIFICATION OF BACTERIA AND FUNGI

Representative colonies were picked from the NA and PDA plates, subcultured and transferred to NA and PDA slants and incubated at 37^0C and 30^0C respectively. Morphological and biochemical tests were done for each isolates and characterized using the schemes of Treagan and Pulliam (1982) and *Bergey's Manual of Determinative Bacteriology* (Bergey and Holt, 1994). Fungal isolates were identified by their colonial, morphological and microscopic characteristics (John and Arandhati, 1995).

2.4. STATISTICAL ANALYSIS

The data obtained were subjected to analysis of variance (ANOVA) using Graph Pad Prism Software, version 5.01. Significant difference between means were determined at $p<0.05$.

3. RESULTS ANALYSIS

3.1. MICROBIAL LOAD OF AFRICAN YAM BEAN YOGHURT

Freshly prepared yam bean yoghurt from dehulled seed had higher total viable and *Lactobacilli* counts. The microbial load of the yam bean yoghurt samples stored at room temperature is shown in Table 1. The total viable count of AYB yoghurt from whole seeds decreased from 8.7×10^7 to 3.2×10^1cfu/g during 14 days of storage while the total viable count was zero on the 21st day of storage. The total viable count for AYB yoghurt from dehulled seeds decreased from 10.4×10^7 to 1.4×10^2cfu/g on the 7th day of storage. Coliforms and *Escherichia coli* were not detected in the yogurt samples throughout the storage period. However, yeasts were detected on the 7th and 14th days of storage. The Lactic acid bacterial counts decreased from 3.4×10^7 to 5.8×10^3cfu/g for AYB yoghurt from whole seeds and from 7.3×10 to 2.3×10^3cfu/g for the sample from dehulled seeds. Lactic acid bacteria were detected throughout the 21days of storage.

Table 1: Microbial load of yam bean yoghurt samples stored at room temperature (29± 2°C)

Storage Period (Days)										
	0		4		7		14		21	
	A	B	A	B	A	B	A	B	A	B
Total Aerobic Count	8.7×10^7	10.4×10^7	4.2×10^5	2.0×10^5	7.8×10^2	1.4×10^2	3.2×10^1	ND	ND	ND
Yeast Count	ND	ND	ND	ND	4.6×10^2	3.2×10^1	5.8×10^3	4.0×10^1	ND	ND
Coliforms	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Escherichia coli	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lactobacilli	3.4×10^7	7.3×10^7	3.8×10^5	1.0×10^5	2.1×10^5	5.5×10^4	1.7×10^4	1.0×10^4	5.8×10^3	2.3×10^3

Key: A = Yam bean yoghurt from whole seed; B = Yam bean yoghurt from dehulled seed; ND = Not detected

The microbial load of yam bean yoghurt samples stored at refrigeration temperature is shown in Table 2. Similar trend of decrease in the total viable count and lactobacilli count for the two yoghurt samples throughout the storage periods were obtained as observed during the room temperature. However, the total viable count and population of *lactobacilli* were higher in the refrigerated sample and yeasts were not detected in these samples during the storage period.

Table 2: Microbial load of yam bean yoghurt samples stored at 4°C

Storage Period (Days)										
	0		4		7		14		21	
	A	B	A	B	A	B	A	B	A	B
Total Aerobic Count	8.0×10^7	11.0×10^7	2.4×10^6	5.0×10^6	6.2×10^5	4.0×10^5	1.1×10^5	4.0×10^5	5.0×10^5	5.2×10^4
Yeast Count	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Coliforms	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Escherichia coli	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lactobacilli	3.0×10^7	7.0×10^7	2×10^6	3.5×10^6	4×10^5	3.6×10^5	1.0×10^5	3.0×10^5	5.0×10^4	5.0×10^4

Key: A = Yam bean yoghurt from whole seed; B = Yam bean yoghurt from dehulled seed; ND = Not detected

4. DISCUSSION

The microbiological quality of AYB yogurt during storage at 29 ± 2^0C and at 4^0C was evaluated. There was a significant difference ($p<0.05$) in pH of yam bean yoghurt from whole seed and yam bean yoghurt from dehulled seed only on the zero day of storage at room temperature. The total viable count and yeast count of yam bean yoghurt from whole and dehulled seeds differed significantly ($p< 0.05$) on days 4, 7 and 14 and days 7 and 14, respectively. The *Lactobacilli* count differed significantly ($p< 0.05$) in the two samples throughout the storage period at room temperature. At refrigeration temperature, the total viable count of yam bean yoghurt from whole and dehulled seeds were significantly different ($p< 0.05$) on days 0, 4, 7 and 14 while the *Lactobacilli* counts were not significantly different.

The high total viable bacterial count explains the acidity of the product and that most of the total viable bacterial count was lactic acid bacteria. Moulds and yeast are the primary contaminants in yoghurt (Dublin-Green and Ibe, 2005). This author also reported that yoghurts stored at 30^0C was spoiled by yeast and became unacceptable after 3-4 days. This illustrates the importance of storage of yoghurt at low temperature in order to increase the shelf life. These microorganisms utilize some of the acid and produce a corresponding decrease in the acidity of yoghurt. The yeast species isolated in the samples stored at room temperature is *Candida albicans*. Oyeleke (2009) also isolated *C. albicans* from nice yoghurt. *Candida albicans* is member of the normal flora of the skin and oral cavity. The successive decrease in microbial load of yoghurt during refrigeration storage has been reported earlier by Laye *et. al.* (1993). According to this author gradual decrease in *Lactobacillus* in plain nonfat yoghurt and this reduces fat oxidation and hydration of protein constituents. Aminigo *et. al.* (2009) also reported a decrease in the lactic acid bacteria count in AYB yoghurt stored at refrigeration temperature for 4 weeks respectively.

5. CONCLUSION

In this study, contamination by yeast characterized the samples stored at room temperature. However, storage of the yogurt at refrigeration temperature beyond three weeks could result in undesirable bacterial counts of the product should have an abundant starter culture. More work should be carried out on how to extend the shelf life of the product stored at room temperature using chemical preservatives as most families either do not have refrigerators or do not have constant power supply.

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4/8/12