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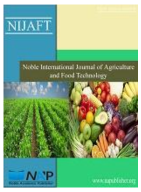

Original Article **Open Access**

ORGANOLEPTIC ATTRIBUTES OF LEGUME-YOGHURT SAMPLES FERMENTED BY LACTIC ACID BACTERIA

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ABSTRACT: The organoleptic attributes of legume-yoghurt fermented by lactic acid bacteria were studied. About 500g of whole legume seeds were sorted, washed, boiled for 30 minutes and then dehulled manually. The dehulled seeds were soaked separately overnight in clean tap water. The seeds were thoroughly washed. The seeds were blended separately in a blender with hot water (seed: water ratio =1:5) till a very smooth consistency was achieved. The resultant slurry from each seed was filtered through cheese cloth to yield the respective legume-milks. About 100ml of the resultant legume milk were pasteurized at 90^{o}C for 15 minutes in triplicates. The first set had no glucose, the second set had 2% glucose while the third had 5% glucose added. Each legume-milk was inoculated with 0.5g of the starter culture and incubated at 42^{o}C for 48 hours in a water bath. After fermentation, the samples were stored for 0-10 day(s) at 4oC and 28^{o}C. Sensory evaluation of three legumes-yoghurt samples with 2% glucose was conducted with dairy yoghurt as a control by 20-member panelist which evaluated the coded samples for appearance, taste, texture, aroma, mouthfeel and overall acceptability using 9-point hedonic scale. Triplicate data obtained were subjected to statistical analysis using SPSS software of version 21. Mean values were determined using One-Way ANOVA and Fisher's Least Significant Difference was used to separate the means at ($p \leq 0.05$). The result showed no significant difference in colour and mouthfeel but significant difference existed for taste, aroma and overall acceptability, with bambara nut yoghurt rated highest.

Keywords: Milks, starter culture, inoculation, incubation, soybean, lima bean, Bambara groundnut.

1. INTRODUCTION

Legumes play an important role in human nutrition as they are rich source of protein, calories, certain minerals and vitamins [1]. They are crops of the family *Leguminosae* that is also called *Fabacae* [2-4]. It is well documented that cereal proteins are deficient in certain essential amino acids, particularly in lysine [5] whereas legumes contained adequate amount of lysine [6]. It is advisable to enhance the protein content of the diet through easily available and accessible plant protein sources especially legumes to improve the nutritional status of the low-income groups of population [7].

Soybean (*Glycine max*) is a rich source of protein and also rich in all essential amino acids. Yet soybean-based foods are not widely acceptable due mainly to the beany flavour and also the belief that they cause flatulence. Preparation of fermented foods using lactic acid bacteria has been suggested to improve their acceptability [8, 9].

Lima bean (*Phaseolus lunatus*) is a legume with limited use. In recent times, plant geneticists have improved the lima beans enormously and a number of early maturing, disease and pest resistant, non-toxic cultivars are widely available commercially. Lima beans are rich in niacin, thiamine and riboflavin. It also contains high levels of potassium, phosphorous, calcium and iron [10].

Bambara groundnut (*Voadezia subterranean*) is also a legume with limited use. It is neglected due to storage induced defects and anti-nutritional factors such as trypsin inhibitors, hemagluttinins, cyanogenic glucosides, anti-coagulants, toxic histones [11]. However, most of these toxins can be reduced to tolerable levels by simple preparative procedures such as fermentation, germination, roasting, soaking in warm water and thorough cooking. Bambara groundnut is a nutritionally balanced seed containing essential amino acids such as isoleucine, leucine, lysine, methionine, phenyl-alanine, threonine and valine. They are also rich in iron [12]. Bambara groundnut are not oil seeds. Hence, they are grown for their edible seeds used as nutritional pulse [13].

Milk is an excellent source of all nutrients except iron and ascorbate. Milk has long been recognized as an important food for infants and growing children. The scarcity of milk supply in developing countries perhaps led to the development of alternative milk from vegetable sources. However, prior to the development of such vegetable milks like soymilk which serve as a less expensive substitute for dairy milk, direct milk consumption as a beverage was not common in Nigeria [14]. The development of milk substitutes extracted from legumes serves as an alternative way of producing an acceptable nutritious food based on vegetable. Vegetable milks can be used for babies in communities where babies are not given dairy milk for ethical reasons such as with vegans or for medical reasons as in milk allergies and galactosemia. Among the sources of vegetable milk, soybean has received very high research attention and more research is still being designed to improve the quality of soymilk [14].

Vegetables do not produce literal milk, like a cow, however there are products made from oil seeds, legumes and cereals that resemble cow milk in appearance and nutrition. Tradition and economic reasons that limit the use of dairy products promote the idea of reducing dairy products as vehicles of the probiotic agents or even replacing with milk from other media, such as cereals, fruits and vegetables [14]. Most prominent of these is soybean milk, its nutritional content is very close to that of cow milk [15]. It can be coagulated to make cheese and fermented to make yoghurt. The production of vegetable milk using legumes and oil seeds is an old technology. However, the technology has been improved to include development of vegetable alternatives to dairy milk, especially in the formulation of infant foods because they are high in protein, mineral and vitamins. Legumes that have been used in vegetable milk production include soybean, cowpeas, winged bean, groundnut and melon seeds; chickpea, pigeon peas, black graw, mung beans, coconut, lupin, peanut, and sunflower seeds [16] and bambara groundnut.

Vegetable milk and vegetable milk products have nutritional benefits for young and old people because of their extreme richness in protein, minerals, essential fatty acids, which are considered to be highly valuable for human nutrition [17]. It is also suitable for both religious (vegetarians) and health (children who are allergic to cow milk protein, people on cholesterol free, lactose free, and dairy free diets) reasons [14]. The current interest in vegetable milk has been motivated by dairy and dairy products being priced too high for the low-income earners [18]. Peanut milk is a low-cost edible product with a high nutritional value. In this regard researchers have focused on products, resulting from fermentation such as yoghurt, buttermilk and ripened cheese analogues [18].

Fermented foods can be included in the category of functional foods, owing to their calcium content and other health promoting components [15]. There is a greater interest in the potential beneficial effects of the fermented milk on health resulting in the increase of available varieties and amount consumed around the world. Dairy products have been used traditionally as vehicles of probiotics in humans [14]. Soybean has received attention from the researchers due to its protein quality. Soymilk is suitable for the growth of the lactic acid bacteria. Several studies have mentioned the production and use of the fermented soymilk drinks as probiotic, mainly soybean yoghurt, which further can be supplemented with oligofructose and inulin [19, 20]. Lima beans and bambara groundnut have potential for use in probiotic beverage owing to their similarity to soybean milk.

Non-dairy probiotic products represent a huge growth potential for the food industry, and may be widely explored through the development of new ingredients, processes, and products. There are a wide variety of traditional non-dairy fermented beverages produced around the world. Much of them are non-alcoholic beverages manufactured with cereals as the main raw material [15]. Nevertheless, fruit juices, desserts and legume-based products can also be used featuring probiotics. The utilization of lactic acid bacteria in preparing soymilk fermentation has received much attention. Little attention has been given to bamabara groundnut milk fermentation by LAB while no attention has been given to fermentation of Lima milk by LAB [15].

Growing awareness of the nutritional benefits of plant-based foods by health conscience consumers, religious reasons, vegetarianism, cholesterol free, lactose free and dairy free diets has led to the increased interest in production of vegetable milk [21]. A lot of attention has been given to soybean milk and its protein isolate beverage since they are considered to be nutritious and healthy [16]. Consequently, soybean and peanuts have been used in a variety of milk-based products including coffee creamers and chocolate milk drink [22]. Legumes and oil seeds have characteristics that make it convenient to combine two or more to obtain an acceptable product. Vegetable milk made from peanut and cowpea blends could be dehydrated to produce an inexpensive dry milk powder [22].

Lactose intolerance, milk allergies, cholesterol content and the increase in consumer vegetarianism are the major drawbacks related to the fermented dairy products [23]. Application of probiotic organisms is at present limited to dairy products especially to yoghurt which may contain residual lactose even after fermentation [23] and can only be afforded by the privileged few in the developing countries, therefore there is need to develop probiotic products from plant sources. The non- dairy probiotic beverages may be made from a variety of raw materials, especially legumes like soybeans, lima beans and bambara groundnut which do not contain lactose.

This study was undertaken to produce legume yoghurts using lactic acid bacteria (LAB) from soybeans, lima beans and bambara groundnut as well as to evaluate consumer acceptability of the different probiotic legume yoghurt. It is therefore important that new studies be carried out to test ingredients, explore more options of media that have not yet been industrially utilised, reengineer products and processes to meet the demands of people with lactose intolerance, milk allergies and vegetarian consumers for new nourishing and palatable cholesterol-free probiotic products [24].

2. MATERIALS AND METHODS

2.1. Materials

Lima beans and bambara groundnut were obtained from a local market in Kaduna. The soybean was obtained from Oil mill market in Port-Harcourt. Commercial freeze-dried yoghurt LAB starter culture (Yogourmet) was obtained from Ariaria market, Aba, Abia State.

2.2. Isolation of Lactic Acid Bacteria

All microbiological processes were carried out in triplicates in a laminar flow to exclude all contaminations. All autoclavable materials were sterilised in an autoclave (Dixons, model ST 1GT UK).

2.3. Preparation of Media and Reagents

All media used were prepared according to specifications and procedures given by the manufacturers. The sterile diluent used was buffered peptone water. MRS agar was used for the selective cultivation of the LAB. The regents used were of analytic grade.

2.4. Isolation of Organisms in the Starter Culture

The pure cultures of LAB were isolated by adding 0.5g of the commercial starter (Yogourmet) to 9.5ml of sterile buffered peptone water (BPW) and homogenised [25]. Six 10-fold dilutions of the homogenates were then prepared and 1ml from each dilution was then subcultured in triplicate, into MRS agars (Biotech, India) used for isolating LAB, and incubated for 48h at 37^{o}C. The isolates were purified by streak-plating on the same medium. Colonies with typical characteristics were randomly selected from plates and tested for Gram staining, cell morphology, catalase and oxidase reaction before further sugar fermentation tests. During the test the cultures were kept in MRS agar slant at refrigeration temperature.

2.5. Identification and Characterisation of Isolates

After obtaining pure culture, following tests were performed for identification purposes:

3. GENERAL MORPHOLOGICAL IDENTIFICATION

3.1. Colonial Identification

Features observed and recorded for colonial identification were shape (whether circular or irregular), size (whether large or small), colour and elevation (whether flat, raised or convex) on the solid medium.

3.2. Gram's Staining

A drop of distilled water was placed on a clean and grease-free microscope slide. A flamed and cooled loop was used to collect a 24h culture and used to make a smear on the water. The smear was air-dried and heat-fixed and then flooded with crystal violet (primary stain). After 60 second, the stain was gently rinsed with water and Lugol's iodine was applied on the smear for another 60 second. This was rinsed with a decolouriser (acetone-alcohol mixture) for 2-3 second, and rinsed with water. The smear was then covered with Safranin (secondary stain) for 60 second and then rinsed with water, carefully blot-dried using filter paper. The smear was mounted on the microscope and viewed using x100 oil immersion lens. Observations were made regarding shape, arrangement of the cells and gram reaction (purple or blue for gram positive and pink or red for gram negative).

3.3. Presence of Spores

A film of 24h old culture of the isolated organism was smeared on a clean slide. This was flooded with 10% aqueous malachite green solution and left to stand for 40-45 minutes. It was washed under running water and flooded with 0.5% aqueous Safranin solution. This was left to stand for 15 seconds and rinsed under running tap. The stained slide was gently blot dried and viewed. Bacteria bodies should stain red while spores should stain green.

3.4. Biochemical Identification

The isolated organisms were also subjected to the following biochemical tests:

3.4.1. Catalase Test

A portion of the colony was transferred to a clean and grease-free glass slide and a single drop of 6% hydrogen peroxide solution was added. Instant effervescence due to the production of oxygen bubbles indicated the presence of the catalase enzyme. Absence of bubble was a negative reaction.

3.4.2. Oxidase Test

A piece of filter paper in a petri-dish was moistened with three drops of oxidase reagent. A glass rod or a platinum loop was used to transfer a colony of the test culture to the filter paper and rubbed on the area moistened with oxidase reagent. The development of a dark purple colouration indicated the production of oxidase. Pink colour changes to purple immediately.

3.4.3. Indole Test

The isolated culture was inoculated in a tube of peptone water containing 0.03% tryptophan and incubated at 30^{o}C. About 0.2ml of Kovac's reagent was added, shaken and allowed for 10min. A pink colouration at the surface indicated the presence of indole.

3.5. Temperature and Salt Tolerance

The growth of bacterial strains at 10°C and 45°C was visually confirmed by the changes in turbidity of MRS broth after 24, 48 and 72 h of incubation. The tolerance of microorganisms to the different levels of salt (4% and 6.5%) was also visually evaluated.

3.6. Carbohydrate Fermentation

Ten millilitre (10ml) of a 10% sugar solution and a 2ml of and red indicator were poured into 90ml of peptone water and sterilised at 121^{o}C for 10min. Five millilitre (5ml) was aseptically pipette into sterile Bijoux bottles with inverted Durham's tubes incorporated to check for gas production and incubated overnight to check for sterility. A pure culture of the test organism was inoculated into the sterile bottles of peptone-sugar solution and incubated at 37^{o}C for up to 7 days. The development of pink colouration indicated production of acid, and displacement of solution with gas in the Durham tube implied gas production. The identities of these isolated bacteria were cross-matched with those present in the standard manuals [26].

3.7. Preparation of the Different Legume Milks

Five hundred grams (500g) of whole soybeans, lima beans and bambara groundnut each, were sorted, washed, boiled separately for 30 minutes and then allowed to cool. They were then dehulled manually, separating the seed coat from the endosperm. The dehulled legumes were soaked separately overnight in clean tap water. After decanting the soak water, the seeds were thoroughly washed. The seeds were blended separately in a blender with the addition of hot water (seed: water ratio = 1:5) till a very smooth consistency is achieved. The resultant slurry from each seed was filtered through cheese cloth to yield the milks of soybeans, lima beans and bambara groundnut respectively (Figure 1).

3.8. Production of the Different Legume Yoghurt Samples and Storage

A one hundred millilitre (100ml) of the resultant legume milk from each seed were pasteurised at 90^{o}C for 15 minutes and transferred into test-tubes in triplicates. The first set had no glucose, the second set had 2% glucose while the third had 5% glucose added. The whole procedure was repeated so as to have two batches of all samples. According to manufacturer's instruction, each test-tube was inoculated with 0.5g of the starter culture, mixed, corked and incubated at a temperature of 42^{o}C for 48 hours in a water bath for fermentation to take place. After fermentation, the samples were stored for 10 days at refrigeration (4^oC) and room temperature (28 ± 3^oC) respectively (Figure 2).

3.9. Sensory Evaluation

Sensory evaluations of the different legume yoghurt samples with 2% glucose were carried out with reference to dairy yoghurt by 20-member panelist consisting of staff and students of the Department of Food Science and Technology, Federal University of Technology, Owerri, Nigeria. The order of presentation of the samples to the panelists was randomized [27]. The panelists were instructed to evaluate the coded samples for appearance, taste, texture, aroma, mouthfeel and overall acceptability. A 9-point hedonic scale quality analysis as described by Peter-Ikechukwu, et al. [9] was used with 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. The panelists were instructed to rinse their mouths with water after tasting every sample and not to make comments during evaluation to prevent influencing other panelists. They were also asked to comment freely on samples on the questionnaires given to them.

3.10. Statistical Analysis

Triplicate data obtained were subjected to statistical analysis using SPSS software of version 21. Mean values were determined and One-Way ANOVA was done as well as Fisher's Least Significant Difference [28] was used to determine for the separation of the means at ($p \leq 0.05$).

4. RESULTS AND DISCUSSION

4.1. Sensory Attributes of the Different Yoghurt Samples

The result of the test for sensory attributes (appearance, mouthfeel, taste, aroma and overall acceptability) of the different yoghurt samples is shown in Table 1. There was no significant difference ($p \leq 0.05$) in appearance and mouthfeel of all samples. However, significant difference existed for taste, aroma and overall acceptability. In terms of taste, there was no significant difference between the dairy yoghurt and bambara groundnut yoghurt and both rated higher followed by soybeans and lima yoghurt. Significant difference existed between the aroma of the samples with dairy yoghurt, bambara yoghurt and soy yoghurt rating better than the lima yoghurt. Also, for the overall acceptability, dairy, bambara and soy yoghurt scored better than lima yoghurt.

The high rating of the dairy yoghurt could be attributed to the fact that the sensory panelists were familiar with the diary yoghurt compared to the soy, Lima and bambara yoghurts. Also, the beany flavour of the soy, Lima and bambara yoghurts may have reduced their acceptability. Similar sensory results have been reported by Lee and Lucey [29], when milk-based yoghurt was compared with soymilk-based yoghurt. It was observed that low acceptability of soymilk yoghurt when evaluated with cow milk yoghurt by sensory panels. The lower rating could be attributed to the off-beany flavour in soymilk yoghurt. The lower the beany flavour, the higher the probability of panelists accepting the yoghurt. Bambara milk yoghurt in this study was more acceptable compared to soy and Lim yoghurt.

5. CONCLUSION

The results showed that legume yoghurts could compete favourably against dairy yoghurt especially Bambara groundnut yoghurt which scored higher overall acceptability than other legume yoghurts. The successful application and consumer acceptability of legume yoghurt has the potential to increase the utilization of these crops and enhance their market value. The legume yoghurts also provide cheap, affordable and refreshing probiotic dairy yoghurt substitute. The use of these legumes as raw materials for probiotics will also create room for variety and sustainability.

Table 1. Mean Values of sensory attributes of the different yoghurt samples

Yoghurt type	Appearance	Mouthfeel	Attributes Taste	Aroma	Overall acceptability
Soy	5.55 ± 2.78^a	6.70 ± 2.02^a	5.79 ± 1.93^b	5.75 ± 1.64^{ab}	5.6 ± 0.82^{ab}
Bambara	5.60 ± 2.17^a	6.55 ± 1.43^a	6.7 ± 1.84^a	6.49 ± 1.80^a	5.8 ± 1.20^a
Lima	5.85 ± 1.38^a	6.60 ± 1.93^a	4.85 ± 1.66^b	5.45 ± 1.90^b	5.2 ± 1.23^b
Dairy	6.20 ± 1.85^a	6.70 ± 2.02^a	7.1 ± 1.65^a	6.7 ± 1.34^a	6.75 ± 0.77^a
LSD	0.94	1.12	0.45	0.12	1.18

Source: Experimental data

Values with the same superscript in the same column are not significantly different at p≤0.05. There was no significant difference in the appearance and mouthfeel of all samples but significant difference existed for taste, aroma and overall acceptability.

Figure 1. Flow Chart for the Production of Legume Milk

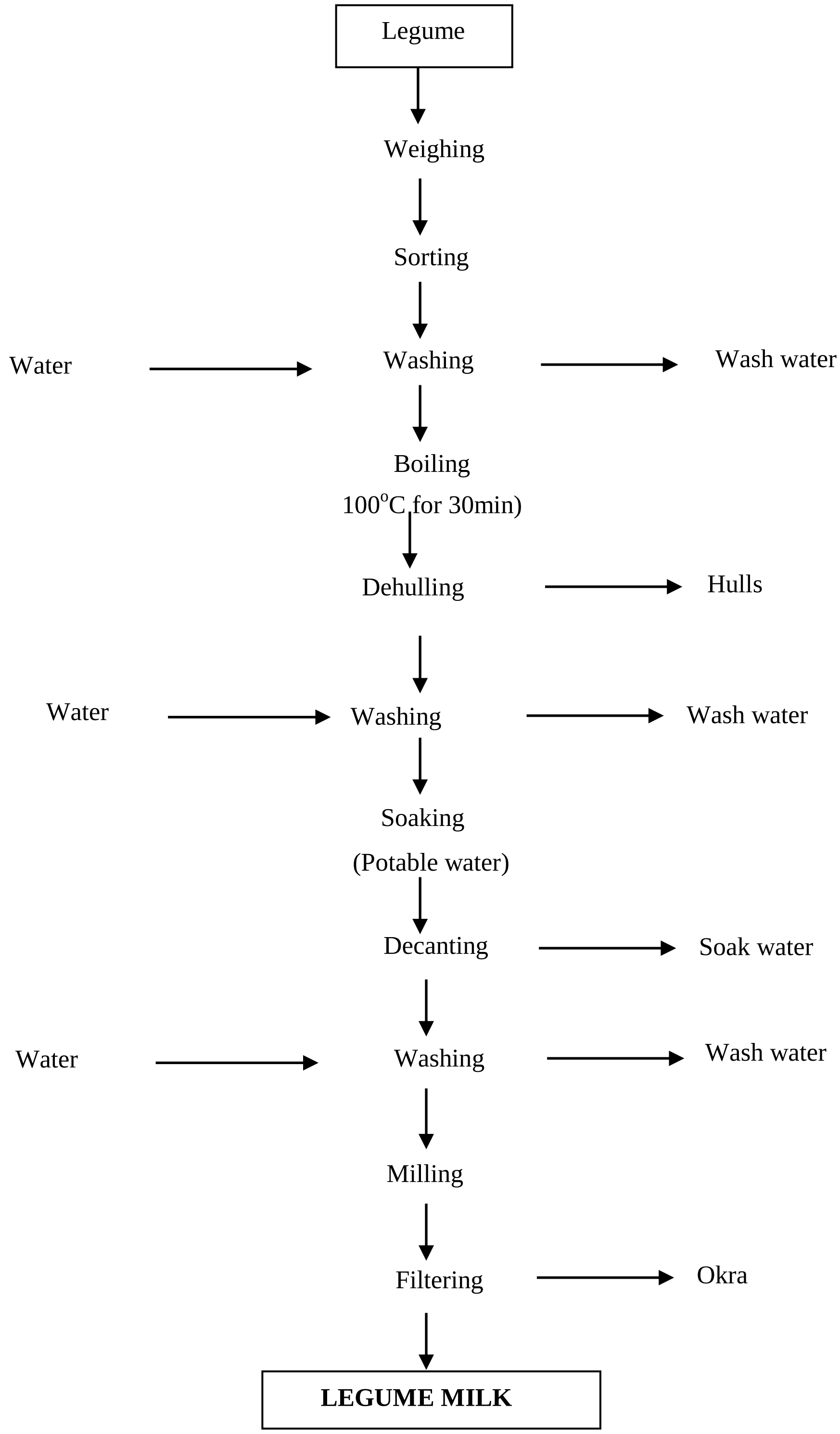

Figure 2. Flow Chart for the Production of Legume Yoghurt

Legume milk

↓

Pasteurisation

(90^{o}C, 15min)

↓

Cooling

(42^{o}C)

↓

Inoculation with starter culture

↓

Incubation

(Water bath, 42^{o}C, 48h)

↓

Interruption

↓

LEGUME YOGHURT

↓

Packaging

↓

Storage

Lima Beans

Soybeans

Bambara Groundnut

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