International Journal of
Science and Engineering Investigations vol. 2, issue 12, January 2013
ISSN: 2251-8843

Production of Soy-Yoghurt by Fermentation of Soymilk with Lactobacillus Isolated from Nunu

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Abstract- The production of soy-yoghurt by fermentation of soymilk with lactobacillus isolated from nunu (a Nigerian indigenous fermented cow-milk) was studied. Soymilk was extracted from whole and dehulled soybean seeds and pasteurized. The fermentation was carried out with both the isolate from nunu and yoghurmet (a commercial yoghurt starter culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) as control. The percentage of soymilk inoculated was 70%, 80% and 100% of the broth. Soy-yoghurt samples produced were subsequently subjected to biochemical and microbiological assays. All the Lactobacilli (LAB) isolated from nunu were gram positive, catalase negative, indole negative, oxidase negative, spore straining negative and produced acid from glucose and lactose. The results demonstrated that addition of nunu to soymilk significantly improved the sensory attributes of soy-yoghurt produced from soymilk. The use of isolate from nunu has the added advantage of reducing the cost of yoghurt starter culture, thereby making soy-yoghurt, a good source of much desired good quality protein cheaper in Nigeria.

Keywords- *Soybean, Soymilk, Soy-yoghurt, Fermentation, Starter Culture and Nunu.*

I. INTRODUCTION

Interest in functional foods has recently increased among consumers due to a greater consciousness of health and nutrition; as well as the need to cure diseases and also the increasing scientific evidence of their effectiveness. Fermented products are a significant part of many indigenous diets. Yoghurt is a Turkish name for a fermented milk product. It is originated by early normadic herdsman, especially in Asia, Southern and Eastern Europe. Yoghurt is made by adding a culture of acid forming bacteria to milk that is usually homogenized, pasteurized and fermented. Yoghurt is defined as a fermented milk product that evolved empirically some centuries ago by allowing naturally contaminated milk to sour at a warm temperature, in the range of 40-50 °C (Ihekoronye and Ngoddy, 1985). The micro-organisms which are used conventionally in this process are referred to as “Starter Culture”. They include Lactobacillus delbrueckii subsp. Bulgaricus and Streptococcus thermophilus. The average size of Lactobacillus bulgaricus ranges from 0.8 to 1.0µm in diameter (Rasic and Kurmann, 1978).

During the fermentation, hydrolysis of the milk proteins occurs, the pH drops, the viscosity increases and bacterial metabolites are produced that contribute to the taste and possibly to the health promoting properties of yoghurt. The sugars are fermented by the bacteria into lactic acid, which causes the formation of the characteristic curd. The acid lowers the pH of the yoghurt and restricts the growth of food poisoning bacteria (putrefactive or pathogenic). Not only is yoghurt a wonderful quick, easy and nutritious snack, but also research evidence point to the fact that milk and yoghurt may actually add years to life as found in some countries where fermented dairy products are a dietary staple. Several health benefits have been reported for traditional yoghurt and this healthy image is enhanced by supplementation with probiotic bacteria (Bakalinsky et al., 1996).

Soymilk is an aqueous extract of soya beans (Glycine max) and is quiet similar in appearance to cow milk (Agure-Dam, 1997). It is commonly characterized as having a beany, grassy or soy flavor, which reportedly can be improved by lactic acid fermentation, as in yoghurt-like products (Jimoh and Kolapo, 2007). Microorganisms possess endogenous β-glucosidases which can be utilized to hydrolyze predominant isoflavone glucosides in soymilk to improve biological activity. It has been reported that probiotic organisms including Bifidobacteria and some other lactic acids hydrolyze isoflavone glycosides into corresponding aglycones (Bordignon et al., 2004; Chien et al., 2006; Sanders, 1997).

Recent reports indicate that some probiotic bacteria could better compete with yoghurt cultures in a soy-based substrate. Soy has been examined as a substrate for the lactobacillus species: L. Casei, L. helveticus, L. fermenti, L. fermentum and L. reuteri (Garro et al., 1999; Murti et al.,1993b; Chumchuere and Robinson, 1999; Garro et al., 2004; Tzortzis et al., 2004). Documented information indicates that soymilk has a significant amount of raffinose and stachyose but does not contain lactose and that lactic acid bacteria (LAB) from different sources are quite different in their efficiencies in soy yoghurt fermentation (Tuitemwong and Tuitemwong, 2003). The problems of soymilk can be improved by lactic fermentation, so production of fermented soymilks such as soy yoghurt is important (Nsofor et al., 1992). A more simplified and cost-effective possibility would be mixing nunu and soymilk to produce quality soy-yoghurt. However, this possibility has not yet been well investigated. Owing to soy-yoghurt's potential immense health benefits, more research targeted at improving its acceptability should be undertaken.

Therefore, our objectives of this study were; to explore the use of soy bean in producing soymilk and production of soy yoghurt by fermentation of soymilk with lactobacillus isolated from nunu.

II. Materials and Methods

Soybean seeds (Variety TGX 526 02D) was obtained from the Nigerian Stored Product Research Institute (NSPRI), Port Harcourt, Rivers State, South-South, Nigeria. Commercially available starter cultures and gelatin were purchased from a local store at Mile 1 market, diobu, Port Harcourt. This study was conducted in the laboratory of the Department of Microbiology, University of Port Harcourt, Choba, Port Harcourt, River State, Nigeria. Soymilk was produced by the method of Mital et al. as reported by Lee et al. (1990)

A. Preparation of Nunu

The Hausa women of Nigeria milk the cows, filling their long gourd containers. The raw cow milk according to Waters-Bayer (1985) was diluted by adding a mixture of water and kuka, a thickening agent made from the acid pith of baobab (*Andansonia digitata*) fruits constitute nunu. The pith of the kuka fruit is rich in vitamins B1 and C, can be mixed with water to serve as a refreshing drink, and is also used to treat intestinal disorders. The water used in dilution of the raw milk came from shallow wells or streams and was not boiled before use.

B. Isolation, characterization and identification of Nunu starter cultures

Fresh nunu samples were left covered on the laboratory bench at ambient temperature (29±2°C) overnight. LAB were isolated from the nunu by serial dilution in 0.1% peptone water and poured into De Mann Rogosa Sharpe (MRS) agar. The isolates were purified by streak-plating on the same medium. Morphological characteristics such as cell shape, colour and arrangement were noted. Biochemical and physiological studies such as catalase and oxidase reaction, type of fermentation, production of ammonia from arginine, growth in 4% NaCl and sugar fermentation profiles were determined using standard methods (Gerhardt et al., 1981; Harrigan and McCance, 1976). The test results were used to identify the organisms by reference to Sneath et al. (1986).

C. Soy-yoghurt production

Two soy-yoghurt premixes were formulated to contain: (i) soy milk (ii) soy milk plus nunu (50:50). Each premix also contained 3% sugar and 0.5% gelatin. Each of the two soy-yoghurt premixes formulated was divided into two portions. Mixtures of premixes, sugar and gelatin were prepared, homogenized and pasteurized as described by Collins et al., (1991). The mixture was subsequently placed in a water bath to cool down to 43°C prior to inoculation with the starter cultures. The first portion of cooled mixtures of each of the two formulations was inoculated with 1% commercial yoghurt culture (50:50) mixture of Lactobacillus bulgaricus and Streptococcus thermophilus as described by Lee et al. (1990). A preculture of each of the two LAB isolates from nunu (Lactobacillus bulgaricus and Streptococcus thermophilus) was prepared; and the mixed culture inoculated into the second portion of cooled mixture of each of the premixes at 5% final volume as described by Murti et al. (1992). All the inoculated premixes were poured into plastic cups and incubated at 43°C to ferment for 12 hours. After incubation, the premixes were cooled in an ice bath, placed in a cabinet at 6±2°C until evaluation within 12 hours.

D. Chemical Analyses

Samples were analyzed for proximate composition using standard methods (AOAC, 1990). The pH was determined using a pH meter while acidity was measured as described by Olubamiwa et al., 2007). A 0.5 ml aliquot of a 1% solution of phenolphthalein in 95% alcohol was added to 10 ml of yoghurt sample. Acidity was measured by titrating the resulting mixture with 0.1N NaOH, expressed as g equivalent lactic acid/100g. All the determinations were carried out in triplicates and mean values were calculated.

E. Sensory Evaluation

The soy-yoghurt samples were kept at 6±2°C until evaluation. A 5-point hedonic scale was used to evaluate the soy-yoghurt samples for acceptability, aroma, taste, color, and mouth feel. The test was conducted by a 15 member panelist. Each panelist was provided with enough privacy to avoid biased assessment.

F. Statistical analysis

The results obtained from proximate and sensory analyses were subjected to analysis of variance (ANOVA). The sensory scores was then subjected to ANOVA using one factor randomized design according to Mahony (1986).

III. Results and Discussion

The pH of the nunu sample evaluated was 5.2 which indicated acidity; nine genera of bacteria were isolated from the samples, kept at 35°C for two days. The nine genera includes lactobacillus (isolates 1,2,3,6,7,8,9), staphylococcus (isolate 4) and streptococcus (isolate 5). The bacteria were isolated on MRS agar and examined for colonial and morphological characterization as shown in Table 1. The morphological characterization indicated that all the isolates were round and large. It also showed that isolates 1,2,6,7,8 and 9 are rod like; isolate 3 is rod like in chains whereas isolates 4 and 5 are nearly spherical. From the biochemical characterization, more accurate results were obtained to characterize the isolated bacteria.

TABLE I. MORPHOLOGICAL CHARACTERISTICS OF ISOLATES.

Isolates	Size	Shape	Color	Elevation	Microscopy
1	Large	Round	Golden yellow	Flat	Rods
2	Large	Round	Brown	Flat	Rods
3	Large	Round	White	Flat	Rods in chains
4	Large	Round	Milky	High convex	Cocci
5	Large	Round	Brown	Raised	Cocci
6	Large	Round	Brown	Flat	Rods
7	Large	Round	Golden yellow	Flat	Rods
8	Large	Round	Golden yellow	Flat	Rods
9	Large	Round	Brown	Flat	Rods

Table 2 showed the bacteria isolates and their probable identity. All the LAB isolates from nunu were gram positive, catalase negative, indole negative, oxidase negative, spore straining negative and produced acid from glucose and lactose. Some of them fermented raffinose and sorbitol while the majority grew in 4% NaCl and at 45°C. Seven of these LAB were homofermentative and majority of the lactic acid bacteria isolated belongs to the genus Lactobacillus. Abdel-Moneim et al. (2006) isolated Lactobacillus constituting 74% of the LAB associated with garris (a Sudanese fermented camel's milk product). Some strains of Lactobacillus that were isolated in this study are identical to those reported in many cultured African dairy products.

TABLE II. BIOCHEMICAL CHARACTERISTICS OF ISOLATES.

Characteristics	1	2	3	4	5	6	7	8	9
Gram Reaction	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-
Spore staining	-	-	-	-	-	-	-	-	-
Growth in 45°C	+	+	+	+	+	+	+	+	+
Growth in 4% NaCl	+	+	+	-	+	+	+	-	+
Growth at 4°C	+	+	+	+	+	+	+	+	+
H_2S Production	-	-	-	-	-	-	-	-	-
Fermentation type	Hm	Hm	Hm	Ht	Ht	Hm	Hm	Hm	Hm
Glucose	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	-
Galactose	+	+	+	+	+	+	+	+	-
Maltose	+	+	+	-	+	+	+	+	+
Frutose	+	+	+	+	+	+	+	+	+
Mannitol	-	-	+	+	-	-	-	+	+
Raffinose	-	-	+	+	-	+	-	+	-
Sorbitol	-	-	+	-	-	+	-	+	-
Xylose	-	-	-	-	-	-	+	-	-

† Table Notes on the next column

† Isolates from nunu only; Hm=Homofermentative; Ht=Heterofermentative. Isolated identities are: 1=Lactobacillus leichmannii; 2=L.casei; 3=L.plantarum; 4=Staphylococcus; 5=Streptococcus thermophilus; 6=L.delbrueckii; 7=L.xylosus; 8=L.bulgaricus; 9= L.fermentum.

Their pH ranges from 5.5-6.5 which indicates acidity. Streptococcus, another lactic acid-producing bacterium was also found in nunu which has an optimum pH of 6.5 and it is a non-spore forming bacteria and a facultative anaerobe. The strains of lactobacillus isolated from nunu were used in the fermentation of soymilk for soy-yoghurt production.

The soymilk produced was observed to have a creamy-yellow color, a pH of 6.5 which indicates slight acidity. Analyses carried out showed that the soymilk consists of water, soybean extracts, sugar and salt; 3-4% protein content and 1.5-2.0% fat. Table 3 shows the composition of the soymilk produced compared to the market standards; that the soymilk is of good quality but the soymilk produced commercially is slightly better due to adoption of good manufacturing, food hygiene practices and standard operating conditions. Twitemwong et al. (1993) found that, soymilk has desirable characteristics as an ingredient for making soy yoghurt because of its high solid content. Angeles and Marth (1971) found yoghurt has titratible acidity of 0.23-0.25% and pH-value of 5.7. Deshpande et al. (2008) found that, soymilk contains total solids (9.8%), fat (2.6%), protein (5.8%), pH value (6.0) and ash (0.6%).

TABLE III. QUALITY ASSESSMENT OF EXTRACTED SOYMILK.

Content	Produced Soymilk	Market standard
Ph	6.5	5.5
Color	Creamy-yellow	Creamy
Protein	3.2% (±1.0)	4%
Fat	2.5% (±0.15)	3%
Moisture	83.7% (±0.66)	80%
Total solids	15%	13%

The soy-yoghurt produced was creamish in color and has a beany flavor. Its pH value (5.2) indicates acidity. It was observed that the soy-yoghurt produced is more acidic than the soymilk from which it was gotten due to the effect of fermentation by the lactic acid-producing bacteria (LAB) that converted the lactose in the soymilk into lactic acid. The high acidity of the soy yoghurt makes it a poor breeding site for pathogenic microorganisms. A comparison of the soy yoghurt produced by fermenting soymilk using a bacteria culture of lactobacillus isolated from nunu with commercial diary yoghurt shows that soy yoghurt produced contains more proteins and moisture but less fat as presented in Table 4. The high amount of protein in the soy yoghurt was due to the fact that soybean is a proteinous food.

TABLE IV. PROXIMATE COMPOSITION OF PRODUCED SOY-YOGHURT AND COMMERCIAL YOGHURT SAMPLE.

Content	Produced Soy-yoghurt	Commercial yoghurt
pH	5.2	4.6
Color	Creamish	Creamish
Protein	3.5% (±0.6)	3.0%
Fat	1.5% (±0.1)	4.0%
Moisture	85.7% (±0.66)	81%
Other materials	7%	12%

The results of the sensory properties of the soy-yoghurt samples are shown in Table 5. Attributes such as color, flavor, viscosity, taste and overall acceptability were evaluated by panelists. Soy-yoghurt samples A and B were rated high in terms of color, taste, viscosity but low in terms of flavor due to the beany flavor associated with soybeans. However, there are no observed differences in terms of overall acceptability for the sample except for the fact that there is high level of deterioration in soymilk based products during storage.

TABLE V. SENSORY EVALUATION OF SOY-YOGHURT SAMPLES

Sensory attributes	A	B
Color	8.1	7.9
Flavor	3.3	5.8
Viscosity	5.4	4.7
Taste	7.2	6.9
Overall Acceptability	6.0	6.2

IV. CONCLUSION

This research showed that soy-yoghurt sample produced from addition of nunu to soymilk competes favorably with yoghurt produced from pure milk. Nutritionally, the soy-yoghurt sample from mixtures of soymilk and nunu met the dietary requirements of pure soy-yoghurt without significant difference when compared with that of literature. However, the flatulence factor and objectionable flavor in soybean products must be reduced or eliminated to enhance acceptability. Despite the findings from this evaluation, there is need for more research on how to mask the beany flavor of soymilk to produce acceptable soy-yoghurt. The choice of appropriate flavor or other additive with low side effects would surely enhance acceptability. It is evident from this study that soy-yoghurt can be produced from blend of soy milk and nunu at various substitutional levels. Therefore, production of soy beverages which are highly consumed by Nigerians and beyond because of the availability of soybeans in commercial quantity is another way of increasing the food value of the crop.

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