SOYBEANS YOGHURT PRODUCTION USING STARTER CULTURE FROM 'NONO'

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ABSTRACT

Yoghurt was prepared from soy-milk equivalent. Soybeans used for milk production were selected, dehulled, blended then mixed with water in ratio 1:6 respectively and filtered with a muslim cloth to obtain milk. On kilogram (1kg) of soybeans used produced soy-milk equivalent of six litres (6L) in moderate dilution. Two genera of bacteria *Lactobacillus* and *Streptococcus* were isolated from 'nono' (naturally fermented cow-milk) using the pour plate technique. The soy-milk equivalent obtained was fermented to yoghurt at 40°C for 14.5 hours using Lactobacillus and *Streptococcus* bacteria as a starter culture in a combination ratio of 1:2. The isolate gave a fine yoghurt like product with a characteristic acid flavour, cream odour and custard-like consistency with a pH 4.36.

Keywords: Soy-beans, Yoghurt and Starter culture.

INTRODUCTION

Yoghurt is a fermented dairy product made from concentrated milk with or without added non fat dry milk. Fermentation is brought about by a mixed culture of bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* The source of milk is usually of animal origin. The product usually contains 12-14% total milk solids and has a soft friable, custard like consistency and a cleans, distinct acid flavour (Lee *et al.*, 1990). The milk meant for yoghurt preparation is first homogenised, heated to a temperature between 85-90°C for 20-30 minutes to pasteurize it and to modify the milk proteins so that they provide the proper viscosity and gelation in the product (Morr, 1985, 1989). It is then inoculated with the yoghurt cultures after cooling to a temperature of 40-45°C. This is incubated for 3-4 hours at 40°C and the yoghurt develops acidity with a resultant drop in pH level.

The product is then cooled and made ready for packaging. Many variations can be made to the procedure. At times certain substances (Skim milk and gelatin) may be added to raise the nutritive value, improve the product body consistency and maintain physical stability respectively.

Soybeans which has been considered a miracle bean by many people is the main source of protein for all of the East Asia, particularly to the vegetarians. It has excellent nutritional properties which make it potentially invaluable as a food for correcting dietary inadequacies of the average Nigerian. Furthermore, its commercial uses in animal feed production and the vegetable oil industry can not be overemphasised (*Kochhar,* 1986).

The cultivation of soybeans in Nigeria has been successfully established but it has been very difficult to cook it in the traditional Nigerian way, so it has never become popular until recently. A greater percentage of the whole produce until now has been exported as cash crop. At present, soybeans is incorporated into so many food formulations of both children and

adults to enhance nutritional value of the foods.

The use of soybeans to produce milk equivalent is popular all over the world (Haumann, 1984). However the soy-milk equivalent produced is characterised by a beany taste or soy flavour which is objectionable to some people. The flavour can be improved by lactic acid fermentation as in yoghurt like products (Angeles and Marth, 1971). Soy-milk equivalent is a suitable medium for growing lactic acid bacteria leading to improved quality of fermented products which resembles yoghurt. Although the milk of animal origin has been successfully fermented into yoghurt by several species of bacteria, vegetable milk equivalent from soybeans has not (Lake and Waterworth, 1980). The process is less predictable because of the biological differences between vegetable and animal milk. Vegetable milk equivalent is low in certain B vitamins (B2 and B6) and drastically low in calcium, fat, carbohydrate and cholesterol. Milk by nature is a highly perishable product and one of the major problem facing the producer has always been the short shelf life of the product. In this regard, there is need to control, improve the method of culturing and subsequent production of yoghurt. This study was conducted to produce yoghurt from soybean milk equivalent using naturally souring bacteria of cow milk ('nono').

MATERIALS AND METHOD

COW MILK: Both fresh and fermented cow milk samples were bought separately from Fulani women in their settlement near Gwallameji Village in Bauchi. Collection was in sterile MacCartney bottles. The samples were taken to the laboratory promptly for further analysis.

Isolation of microorganisms from 'nono'

Serial dilutions were made of the sample. The pour plate technique was used in plating our 1ml of the various dilutions (10^{-1} to 10^{-3}) into sterile petri dishes in triplicates. Molten nutrient agar was added and the plates were swirled gently to mix the contents. They were then allowed to set and incubated at 37°C for 24 hours.

Isolation of pure cultures of isolates.

Different distinct colonies from the incubated plates were picked and subculture on fresh nutrient agar media using sterile wire loop. These were incubated at 37°C for 24 hours for colony development/growth.

Characterization and identification of isolates.

The standard procedures of Collins and Lyne, (1987) were adapted. The cultural characteristics such as shape, size, pigmentation, opacity and nature of margins were observed and recorded. This was followed by microscopic examination of cell types, arrangement, and Gram's reaction as well as motility attribute. Following this was the biochemical behaviour of the isolates as regard sugar and other chemicals utilization and the final confirmation of genera of organisms using Bergey's manual of Determinative Bacteriology (Buchanan and Gibbons, 1975).

Soybeans

Soybeans seeds of yellow variety were purchased from Yelwa market within Bauchi and kept in clean polytene bags at room temperature until use.

Soy-milk equivalent

This was prepared using the method of Wilson, (1984). One kilogram (1kg) of soybeans were sorted out, cleaned and washed in tap water. The beans were later soaked for 18 hours then drained. They were decoated, washed well with distilled water and heated in boiling distilled water at 96 - 100°C for 15 minutes then ground into a paste using a blender. The resulting paste was diluted with distilled water in the ratio of 1:6. The suspension was filtered using a cheese cloth and put into a flask.

Yoghurt preparation

To the prepared soy-milk equivalent was added lactose and sucrose sugar (7.5% and 5.0% W/V respectively and mixed thoroughly. This was pasteurised at 65°C for 30 minutes then cooled to 40 - 45°C. Fifty millilitre (50ml) quantity of the soy-milk equivalent was put in different sterilised conical flasks and inoculated with 2% (V/V) of 24 hours old culture inoculum of the different isolates from fermented cow milk. In addition, different combination of the inocula were made in equal and unequal ratios. *Lactobacillus* and *Streptococcus* were combined in 1:1, 2:1 and 1:2 ratios. *Lactobacillus* and *Staphylococcus, Lactobacillus* and *Bacillus, Streptococcus* and *Staphylococcus* as well as *Bacillus* and *Staphylococcus* were all in 1:1 ratio. *Lactobacillus, Bacillus* and *Streptococcus, Lactobacillus, Streptococcus* and *Staphylococcus, Lactobacillus, Staphylococcus* and *Bacillus* were in 1:1:1 ratio. Incubation of the inoculated soy-milk equivalent was at 40°C for 15 hours.

Chemical analysis

Moisture content of fresh cow milk and soy-milk equivalent were determined by the Food and Agricultural Organisation (FAO) 1988. Fat, protein and ash content determination was by the methods of Harold *et al.,* (1991). And the carbohydrate value was gotten by adding the percentages of water, fat, protein and ash then subtracting the value from 100%. pH determination was by using a pH meter (mini - 80 Tacussel electronique) standardized with buffer solution of pH meter 4.0 and 9.2. Values obtained were recorded as the pointer became steady.

Titrable acidicity

The procedure of Speck, (1984) was adopted and total acidity was expressed as lactic acid.

Sensory taste

Sensory evaluations were made by a taste panel of 14 members. Two samples were used, one as a standard ("Fan" yoghurt) and the other was the product. They were coded A & B. The samples were evaluated for characteristics such as taste, colour, odour and consistency. Each panel member was asked to indicate his/her degree of likeness for each attribute on the product. A nine point Hedonic scale test was used as described by Ihekoronye and Ngoddy, (1985).

In the test a score of 9 indicates like extremely while I indicates dislike extremely. The other options in between these two were scored accordingly.

RESULTS AND DISCUSSION

Results in Table I shows that soy-milk equivalent like animal milk contain proteins, fat, carbohydrates and minerals. The proportions however are not the same. Fresh cow milk has a higher quantity of all the constituents determined.

Table I percentage chemical composition of fresh cow milk and soy-milk equivalent.

CONSTITUENT	FRESH COW MILK	SOY-MILK EQUIVALENT
Water	87.2	92.47
Protein	3.5	3.05
Fat	3.6	1.90
Ash	0.7	0.58
Carbohydrate	5.0	2.00
pH	5.4	6.26
Titrable acidity	0.75g	0.11

Differences could arise due to the difference in source of origin (Lillian, 1978, Kirk and Sawyer, 1991). The protein content of soy-milk equivalent is almost same with that of cow milk despite the high dilution of the former. This explains why it can be used as a supplement or substitute for cow milk. The soy-milk equivalent has added advantage of low cost. Four genera of microorganisms were isolated from the fermented cow milk *Lactobacillus, Bacillus, Streptococcus* and *Staphylococcus.* This agrees with earlier findings of Olawuyi, (1987). The quantity of 6 litress of soy-milk equivalent obtained only from one kilogram of the dried beans shows how favourable the product can compete with cow milk. If more work is done on its market acceptability, its ready availability could enhance its substitutive power as a commercial source of milk.

The four genera of bacteria and their combinations inoculated into soy-milk equivalent for yoghurt preparations gave a variety of products at end of the incubation period. The best soy-yoghurt was obtained with the use of *Lactobacillus* and *Streptococcus* species combinations in the ratio of 1:2.

It had a characteristic lactic acid flavour and a pH of 4.36 (see table 2) Though all the organisms can grow in soy-milk equivalent not all produced the desired products. Their ability to utilize soy-milk equivalent stems from its rich nutritional value and this agrees with Chang and Stone, (1990). The bacteria involved might have enzyme systems capable of metabolizing the various components of the milk. Different products with their characteristic odours account for the diversity in the types of enzymes released and the pattern of breakdown. The introduction of starter culture only will give rise to the desired product. Both the pH and the titrable acidity of soy-yoghurt produced are within the range considered optimal for aroma development. The best yoghurt product from the combination ratio of 1:2 *Lactobacillus* and *Streptococcus* scored 54% as against the 64% commercially available standard ("Fan" yoghurt). This compares favourably with the commercial product made from the animal milk. The colour, odour consistency and taste were all rated very high.

TABLE 2: CHARACTERISTICS OF SOY-YOGHURT PRODUCED BY DIFFERENT SPECIES OF BACTERIA AND THEIR COMBINATIONS

BACTERIAL SPECIES	COMBIN-ATION RATIO	ODOUR	FLAVOUR	CONSIST-ENCY	pH	TITRAT-ABLE ACIDITY
Lactobacillus	single	cream	slight acid	custard like	5.84	0.67
Streptococcus	single	cream	acid	"	5.01	0.70
Staphylococcus	single	pungent	flavour	"	-	-
Bacillus	single	"	off-flavour	"	-	-
Lactobacillus and Streptococcus	1:1	cream	"	"	4.26	1.02
Lactobacillus and Streptococcus	2:1	"	acid	"	4.30	1.03
Lactobacillus and Streptococcus	1:2	"	flavour	"	4.36	1.01
Lactobacillus and Staphylococcus	1:1	pungent	"	"	-	-
Lactobacillus and Bacillus	1:1	"	"	"	-	-
Streptococcus and Staphylococcus	1:1	"	off-flavour	"	-	-
Bacillus and Staphylococcus	1:1	"	"	"	-	-
Lactobacillus Bacillus and Streptococcus	1:1:1	"	"	"	-	-
Lactobacillus Streptococcus and Staphylococcus	1:1:1	"	"	"	-	-
Lactobacillus Staphylococcus and Bacillus	1:1:1	"	"	"	-	-
"Fan" yoghurt*	-	cream	mildly sour	smooth	4.5	0.93

KEY: - not determined
* standard.

Though the soy-milk equivalent based yoghurt compares favourably with the commercially available animal milk yoghurt, more work is needed to determine the correct proportion of premix consistuents to make up for the absence of the other constituents not found in enough quantities is soy-milk equivalent.

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