Assessment of Physico-chemical Characteristics, Viability and Inhibitory Effect of Bifidobacteria in Soymilk

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Abstract

The study investigated viability of *Bifidobacteria* isolates from breast-fed infants in soymilk with the aim of producing a beverage with health- improving potentials from an affordable source.

Strains of *Bifidobacteria* spp isolated from breast-fed babies were identified on the basis of morphological and biochemical characteristics. The pure isolates were inoculated into sterile soymilk and their viability was studied at ambient (28 ± 2^{o}C) and refrigeration temperatures (4 ± 1^{o}C) The bifidobacteria isolates were identified as *B. adolescentis* and *Bifidobacterium longum*. The inhibitory effects of the *Bifidobacteria* isolates were tested on *Escherichia coli* and *Staphylococcus aureus*. *B. longum* was found to be viable for 35 days and inhibited *Escherichia coli* better than *Staphylococcus aureus*. Changes in the physico-chemical characteristics of the inoculated soymilk were also monitored over the storage period.

Crude protein increased from 1.76 to 2.14% and 1.96% in soymilk samples stored at ambient and refrigeration temperatures respectively.

The study has shown that *B. longum* which is supported by soymilk exhibits antimicrobial activity against tested food borne pathogens – indicating that probiotic soymilk could be produced from *B. longum* and soymilk.

Keywords: Ambient, Refrigeration, *Bifidobacterium*, Inhibitory, Soymilk, Viability

1. Introduction

Probiotics are live microorganisms which when ingested enhance the well-being of the host through their effects on the intestinal microflora (Havenaar & Veld, 1992; Aunty *et al.,* 2001). Other reported positive effects of probiotics include digestion of lactose, synthesis of vitamins and lowering of serum cholesterol levels (Aramide *et al.,* 2009). They contribute to biological defense mechanisms, prevention of diseases, recovery from diseases, and control of physical conditions and influence the ageing process (Patidar & Prajapati, 1997). From the foregoing, incorporation of probiotic bacteria with scientifically supported health claims in foods has great potentials for improving the quality of life.

The most widely used probiotics belong to the genera *Lactobacillus* and *Bifidobacterium* (Bowley, 2005). *Bifidobacterium* is the dominant bacteria in the intestinal microflora of breast-fed infants (Abe, 2004). For a probiotic bacteria to benefit human health it must be able to withstand the manufacturing process without losing its viability and functionality when incorporated into food products (Saarela *et al.*, 2000). Dairy products have been employed in the food industry and embraced by many consumers as vehicle of delivering probiotic microorganisms. Various types of fermented milk products including bifidus milk, and bifidus-acidophilus milk are examples of popular *probioticated* products obtained with Bifidobacteria (Kurman & Rasic, 1993; Patidar & Prajapati, 1997).

Currently, efforts are being made to use plant sources such as roselle extract, ginger and garlic extract as substrates for cultivating probiotics (Aramide *et al.*, 2009, Adeniran *et al.*, 2010). Soy products have been suggested as the best substitute for dairy products (Tsangali *et al.*, 2002). Among the reasons for this are low cost, being lactose-free, non-allergenecity and low technology required. It is reported that soymilk can be produced for about one third to one half the cost of cow's milk and a unit area of the land can produce about 10 times as much soymilk per year as dairy milk (Samona, 1993). Thus it is important that the developing countries begin to think of partially

replacing the increasingly expensive dairy products with high quality soymilk (Nsofor & Osuji, 1997). In addition, lactose intolerant people feel discomfort and pain, usually accompanied by diarrhea when they consume dairy milk (Poskitt, 1993). Soymilk can be consumed by these groups of people since it contains no lactose and has been reported to be an excellent dairy milk substitute (Nsofor & Osuji, 1997; Omoni & Aluko, 2005).

Furthermore, the other documented health advantage of consuming soymilk is its being cardio protective. That is, it contains beneficial cholesterol, not the bad cholesterol that can cause thrombosis and high blood pressure. A diet high in soy foods has been associated with reduced risk of several types of cancer, such as breast, endometrial and prostate. Soy isoflavones' antioxidant properties likely contribute to soy foods' anti-cancer effects by reducing free radical damage to fats (lipid peroxidation) and DNA. Soy isoflavones may also induce Phase II liver enzymes (such as glutathione-s-transferase and quinone reductase), which help the body eliminate the toxic byproducts (aromatic hydrocarbons) produced when meats are charbroiled (Azadbakht *et al*., 2003). Soymilk has also been found to be capable of reducing Type 2 diabetes, kidney and health diseases (Villegas *et al*., 2008)

This study therefore examined the viability of *Bifidobacteria* species isolated from breast-fed babies in soymilk for five weeks. The isolates were earlier characterized and their inhibitory effects on potentially pathogenic organisms in soymilk evaluated. Accompanying changes in physic-chemical characteristics of the inoculated soymilk were also monitored over a period of storage.

2. Materials and Methods

Soybeans were purchased from the main market, Ile-Ife, Nigeria. The pathogenic organisms, *Escherichia coli* (type strain NCIB 86) and *Staphylococcus aureus* (type strain NCIB 8588) used were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.1 Methods

The experiment included isolation of *Bifidobacteria* and *Lactobacilli* spp from faecal samples from breast-fed babies, characterization of the microbes, preparation of soymilk, inoculation of the milk with the bacterial isolates and determination of inhibitory effect of probiotic microbe with pathogenic organisms.

2.2 Preparation of soymilk

Soybeans were processed into milk using the method shown in flow chart of Figure 1. Subsequently, 27ml of the prepared soymilk was dispensed into 30ml screw capped test tubes and heat treated in an autoclave at 115 ^{0}C for 20*mins*.

2.3 Microbiological analyses

Media for cultivation, isolation and identification of bacterial isolates were of analytical grade (Oxoid, England); and were prepared using standard methods. All media for bacteria cultivation and identification were sterilized by autoclaving at 121 ^{0}C for 15*mins*.

2.3.1 Isolation of *Bifidobacterium* and *lactobacillus* species from faecal samples

Faecal samples (20 samples) from healthy breast fed babies were collected from the Children's immunization section of the Obafemi Awolowo University, Teaching Hospital complex, IleIfe, Nigeria into sterile McCartney bottles and immediately transported to the laboratory.

Isolation of *Bifidobacteria* was done by mixing one gram of each of the faecal samples with 9ml of reinforced clostridial medium (a diluent for anaerobes) (Harrigan & McCance, 1976; Harrigan, 1998). Serial dilutions were done and pour plates were made with a selective medium, trypticase peptone yeast extract agar (TYP) supplemented with 0.1% sodium thioglycholate and 0.5% propionic acid (Olutiola *et al*., 1991; Yusof *et al*., 2000). Plates were incubated at 37 ^{0}C for 72*h* under anaerobic condition using anaerobic jar and gas generating kit (Oxoid; Basing stoke, U.K). From the diluted samples, Lactobacilli were isolated by mixing 1 ml of samples with de Man Rogosa and Sharpe agar, (MRS) containing 1% Sodium azide. The 1% sodium azide provided micro aerophilic condition (Harrigan & McCance, 1976; Brankovi & Baras, 2001). Plates were incubated at 37 ^{0}C for 72*h* under aerobic conditions.

2.3.2 Identification of bacterial isolates

Representative colonies on trypticase peptone yeast extract agar and de Man Rogosa and Sharpe agar were picked on the basis of their cultural characteristics, and purified by repeated streaking on the fresh sterile media plates and incubated. Pure cultures obtained were coded and transferred to agar stabs and slants respectively and stored in the refrigerator until required for identification. The bacteria isolates were identified based on their cultural, morphological and biochemical characteristics following the schemes of Harrigan and McCance (1976) and

Sgorbati *et al.* (1995). Characterization was based on cultural and microscopic morphology, Gram's reaction, growth characteristics, and selected biochemical tests including catalase test, nitrate reduction, ammonia reduction, and sugar fermentation test.

2.3.3 Preparation of test samples for inoculation

(a) Prepared sterile soymilk (27ml) was dispensed into each of the test tubes and each tube was inoculated with 3ml of pure *Bifidobacteria* culture at 10% (v/v) concentration. A set of the samples was stored at ambient temperature (28 ± 2 ^{o}C) while the other set was refrigerated (4 ± 1^{o}C) for viability test.

(b) For the antagonism test, equal number of tubes containing 28ml of sterile soymilk was each inoculated with 1ml of the *Bifidobacteria* culture and 1ml of 24*h* old culture of *E. coli.* The test samples were stored at ambient temperature.

(c) Another set of tubes containing 28ml of sterile soymilk were each inoculated with1ml of the *Bifidobacteria* culture and 1ml of 24*h* old culture of *Staphylococcus aureus*. Samples were kept at ambient temperature.

(d) Equal number of tubes as in (b and c) above containing the sterile soymilk was each inoculated with 1ml 24*h* old culture of *E. coli.* This served as control samples for the test samples in (b) above. The control samples were also stored at ambient temperature.

(e) The same number of tubes with each containing 28ml of sterile soymilk as in (d) above was each inoculated with 1ml of 24*h* culture of *Staphylococcus aureus.* This served as control for (d) above. Samples were kept at ambient temperature.

2.3.4 Determination of the viability of *Bifidobacteria* isolates in soymilk

Counts of the *Bifidobacteria* were determined weekly for 5 weeks from inoculated soymilk samples stored at both ambient and refrigeration temperatures. From each tube, 1ml was pipetted into 9ml of reinforced clostridial medium, mixed thoroughly and serial dilutions of the mixture were made and the microbe enumerated using pour plate method. Diluted samples were poured trypticase peptone yeast extract agar supplemented with 0.1% ascorbic acid and 0.5% propionic acid (Hoover, 1999). The plates were incubated at 37 ^{0}C under anaerobic condition for 72*h* and the colony count of the *Bifidobacteria* colonies was done using a Gallenkamp colony counter (CNW-300 model) (Harrigan & McCance, 1976; Harrigan, 1998)

2.3.5 B*In vitro* antagonism of *Bifidobacteria* isolates and pathogenic strains in soymilk

The inhibiting effect of the selected *Bifidobacteria* species on food borne bacterial pathogens was assayed by the broth culture method described by Visser & Holzapfel (1992) and Kobayashi *et al.,* (2003). Samples were taken from each test tube of soymilk inoculated with 1ml of *Bifidobacteria* culture and 1ml each of 24*h* cultures of *E. coli* and *S. aureus* respectively, and from each tube of the soymilk samples inoculated with 1ml each of the pure cultures of the pathogenic organisms all stored on the shelf at room temperature, weekly for 5 weeks for counts of the pathogens.

2.3.5.1 *Escherichia coli*

Counts for *E. coli* was accomplished by mixing 1ml of the soymilk (containing Bifidobacteria culture and 1ml each of 24*h* cultures of *E. coli*) into 9ml of 0.1% peptone water diluent (Harrigan & McCance, 1976) and mixed thoroughly. Serial dilution of each of the inoculated samples was carried out. From each diluted sample, 1ml was pipetted into each plate and about 20ml of MacConkey agar was introduced into each (Harrigan and McCance, 1976; Olutiola *et al*., 1991). The plates were incubated aerobically at 37 ^{0}C and observed after 24*h* (Yusof *et al.*, 2000). Counts were achieved using a Gallenkamp colony counter (CNW-300 model).

2.3.5.2 *Staphylococcus aureus*

Counts of *S. aureus* was achieved by mixing 1ml of the sample (containing *Bifidobacteria* culture and 1ml each of 24*h* cultures of *S. aureus* overnight culture of *S. aureus* into 9ml of a 0.1% peptone water diluent (Harrigan and McCance, 1976; Harrigan, 1998) and mixed thoroughly. Serial dilution of the inoculated samples was carried out and pour plates made with Mannitol Salt agar. The plates were incubated aerobically at 37 ^{0}C and observed after 24*h*. Counts were done using a Gallenkamp colony counter (CNW-300 model).

2.4 Determination

2.4.1 pH, Titratable acidity and total solids determination

The method of AOAC (2000) was employed in determination of pH, Titratable acidity (TTA) and Total Solids

2.4.2 Determination of Amino Nitrogen

Amino nitrogen was determined using the Formol titration method (Frais, 1972). The formaldehyde used in this analysis was first neutralized with standardized 0.1M sodium hydroxide solution. Five ml of each soymilk sample were put in conical flasks and two to three drops phenolphthalein added. These were then neutralized with standardized 0.1M sodium hydroxide solution, adding the alkali dropwise until the faintest permanent pink color was produced. Six milliliters of the neutralized formaldehyde solution were added to each of the neutralized soymilk samples. The disappearance of the pink color was observed, and then the contents of the flasks were titrated against the standard sodium hydroxide solution until a pink colour was produced as before, the titre values were recorded and used in calculating the amino nitrogen present in each sample.

2.4.3 Determination of total reducing sugar.

Total reducing sugar was determined by the use of Dinitrosalicylic reagent (Miller, 1959 & Adeniran *et al*., 2008). The soymilk samples were diluted to 4 times its volume with distilled water and filtered through a paper filter placed in a funnel. One ml of each of the sample's filtrate was put in test tubes and 1ml distilled water was put in another test tube as blank after which 1ml of DNSA was added to each of the test tubes and boiled for 5mins. After boiling, 10 mls of distilled water were added to each tube and the absorbance read at 540 nm in a Spectrophotometer (CECIL CE 3041 model). The reducing sugar concentrations of the samples were read from a calibration curve prepared by measuring the absorbance of varying concentrations of glucose and DNSA reagent at 540nm.

2.4.4 Determination of Proximate Composition of inoculated soymilk

AOAC (2000) method was used to determine the proximate composition of the soymilk after inoculation with *B. longum* and during subsequent storage at ambient and refrigerated temperatures.

2.5 Statistical analysis

The values obtained from each of the analyses were means of triplicate readings. Origin Pro (1999-20020) computer package was employed to analyze the experimental data generated.

3. Results and Discussion

3.1 Identification of human intestinal probiotic strains

Strains of *Bifidobacteria* isolated from fecal samples of breast fed babies were characterized as shown in Table 1. They were identified on the basis of their unique morphology of V- or Y-shape or being multiple branched rods. This is an important differentiating property between the two related genera *Bifidobacterium* and *Lactobacillus* as (Mitsuoka, 1992; Sgorbati *et al*., 1995; Brankovi & Baras, 2001). The bifidobacteria isolates were identified as *Bifidobacterium longum* and *Bifidobacterium adolescentis* based on their fermentation characteristics. Both species have been reported to be a part of the faecal bacteria population in breast fed babies (Mitsuoka, 1992; Sgorbati *et al*., 1995). Most of the Bifidobacteria isolates in this study were identified as *Bifidobacterium longum*. It is considered the commonest species of *Bifidobacteria* found both in infant and adult faecal material (Sgorbati *et al.*, 1995).

The DNA of *B. longum* is said to be 50 – 76% related to that of *Bifidobacterium infantis*. This close genetic relationship is reflected in their similar fermentation activity (Sgorbati *et al.*, 1995) and the identification of strains belonging to one of the two species is reported to remain a difficult task (Sgorbati *et al*., 1995). For identification, Scardovi (1986) suggested that strains able to ferment arabinose and melezitose be regarded as *B. longum* while strains that cannot ferment arabinose be held as *B. infantis.*

The *Lactobacillus* strains isolated were characterized on basis of their morphology and fermentation profile as *Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus salivarius* (Mitsuoka, 1992).

3.2 Viability of the Bifidobacteria isolates in soymilk.

Bifidobacterium longum, which was inoculated into the sterile soymilk, proliferated in soymilk as shown in Figure 2. An initial increase in population was observed in samples stored at both storage temperatures; this was followed by a decrease from the 21st day onward. This observation agrees with the finding of Gilliland *et al.* (2002) in which the growth of *B. longum* was monitored in a fermented milk product over a period of 35 days. This decrease after 21 days could be explained as being the result of diminishing nutrient supply (Tsangalis *et al*., 2002) and decreasing pH below values required for optimum growth (Hoover, 1999). Aramide *et al.,* (2009) found *Lactobacillus acidophilus* and *L. plantarum* viable in roselle extract after 27 days of storage at ambient temperature while Adeniran *et al.,* (2010) also reported that after 5 weeks of storage of inoculated samples at

ambient temperature, *L. bulgaricus* and *L. plantarum* were still viable in ginger extract. Finding of the present study is in agreement with similar studies on *Lactobacillus* spp on plant-based beverages.

The rate of population reduction was lower in the refrigerated samples as expected. A slight decrease in bacteria population during the first seven days of refrigeration was also observed. This could be due to the sudden cold storage (Hoover, 1999; Prescott *et al*., 1999, Adeniran *et al.*, 2010) or cold shock effects on the microbial cells, a phenomenon called the lag phase. This also accounted for the lower Bifidobacteria counts observed when compared to samples stored at room temperature especially at periods of maximum microbial growth, day 14 and 21. Generally, the growth of microorganisms and in particular Bifidobacteria is known to slow down at low temperatures (Hoover, 1999; Gilliland *et al*., 2002).

The higher counts in the refrigerated samples on the 28th and 35th day could be as a result of residual nutrients in the soymilk which was being slowly used up by the growing microbial population and also as the result of the slightly higher pH values which were within the pH values for the optimum growth of Bifidobacteria (Hoover, 1999; Gilliland *et al*., 2002). The values were further subjected to statistical analysis and the correlation between storage at room and refrigeration temperatures were found not to be statistically significant at 5% level. This suggests that the soymilk *probioticated* with *B. longum* could be stored at both temperatures without significant reduction in the bacterial counts.

B. *adolescentis*, on the other hand, was observed to be viable in the inoculated soymilk for less than 3 days at ambient and refrigeration temperatures. No viable count of the isolate was recorded at 3rd day of incubation in the soymilk samples. The reason for the non-proliferation of this organism in soymilk could be due to its inability to metabolize the predominant sugars in the soymilk, which were mainly raffinose and stachyose requiring α-galactosidase activity for metabolism (Hoover, 1999; Tsangalis *et al*., 2002). Also, this particular isolate was found to be a strict anaerobe. This could have caused its non-proliferation in the soymilk since steps were not taken to ensure a low oxidation – reduction potential in the medium during storage (Hoover, 1999; Gilliland *et al*., 2002).

3.3 Antimicrobial effect of the isolate Bifidobacterium longu

Presented in Figure 3 is the result of viable counts of food borne pathogens which were cultured with B. longum in soymilk, stored at ambient temperature. This is the temperature at which pathogens grow and produce toxins (Prescott et al., 1999). This result reflects the inhibitory effects of the test probiotic strain against food borne pathogens E. coli and S. aureus. According to earlier findings, Bifidobacteria have been known to help in maintaining healthy functioning of the intestinal tract directly through antimicrobial activity (Danone, 1997). The results obtained in this study show that the pathogens E. coli and S. aureus had higher counts when grown as pure cultures in the soymilk than when grown in combination with the probiotic isolate, throughout the duration of storage. This is in agreement with the results of a study which showed a reduction, and finally the extinction of a plant pathogen treated with the probiotic strain L. plantarum (Visser & Holzapfel, 1992).

Figure 3 also shows that the growth of E.coli was better inhibited compared to the growth of S. aureus. Escherichia coli has been reported to be strongly inhibited by acetic acid, especially by those produced by some strains of the probiotic species B. longum (Kobayashi *et al.*, 2003; Ibrahim *et al.*, 2005) and could be inferred that the inhibitory effect on E.coli observed in Figure 3 could be due to the presence of organic acid (likely acetic acid) produced by the probiotic strain in the soymilk. As with other short chain fatty acids the undissociated form of the molecule acetic acid mediates the antimicrobial effect by collapsing the electrochemical proton gradient causing bacteriostasis and eventual death of susceptible bacteria. Acetic acid and propionic acid have higher pKa values than lactic acid and therefore a higher undissociated ratio at a given pH. This is reported to be one reason for their increased antimicrobial efficacy compared to lactic acid (Earnshaw, 1992).

For sample containing the probiotic strain and the pathogen *S. aureus,* the degree of inhibition by the probiotic strain was observed to be lower and the pathogen remained viable throughout storage duration while for the sample containing the probiotic strain and the pathogen *E*. *coli*, the pathogen remained viable until the 21st day of storage when it was completely inhibited. Findings of Bowley (2005); Ibrahim *et al.* (2005); Aramide *et al.* (2009) and Adeniran *et al*. (2010) affirmed that different probiotic strains exhibit different degree of inhibition against different types of pathogens.

3.4 pH and titratable acidity values of B. longum inoculated soymilk

From Table 2, it can be seen that the pH values of samples stored at ambient temperature showed a decrease from 5.72 on the first day of storage to pH 4.58 on the 7th day compared to refrigerated samples which showed a gradual decrease from 5.72 on the first day of storage to 5.63 on the 7th day. At the end of storage period, the pH was 4.36 and 5.35 respectively for samples stored at ambient and refrigeration temperatures. There was no appreciable

change in the pH of the control samples by the 35th day. The decrease in pH of the test samples was probably due to the production of short chain carboxylic acid such as acetic acid and lactic acid, the major fermentation products of the genus *Bifidobacterium* (Yusof *et al*., 2000; Kobayashi *et al*., 2001; Brankovi & Baras, 2001; Tsangalis *et al*., 2002). The difference in the rate of decrease in the pH of samples stored at ambient temperature compared to those refrigerated was due to relatively higher level of microbial growth and hence increased acid production (Prescott *et al*., 1999). The pH was not low enough to cause a permanent change in the consistency of the soymilk by the end of the storage period.

Result of TTA indicates that that the acidity of the inoculated samples increased with storage time.

3.5 Total solid content

The total solids decreased from 5.16 to 4.43% at the end of the storage period for samples stored at ambient temperature and from 5.16 to 4.50 for samples stored at refrigeration temperature (Table 2). This could be attributed to the uptake of these solids by the growing microbial population (Nsofor *et al*., 1997; Rani & Khetarpaul, 1999).

3.6 Amino Nitrogen and crude protein content

Table 3 shows the changes in amino nitrogen and crude protein content of soymilk samples inoculated with *B. longum* stored at ambient and refrigeration temperatures. An initial increase of the amino nitrogen content was observed from 0.18 to 0.22 mg ml^{-1} in the two sets of *probioticated* soymilk samples. This could be due to partial breakdown of protein to peptide and amino acid (Rani & Khetarpaul, 1999). Thereafter, the amino nitrogen level was observed to be quite stable. It is likely that the amino nitrogen taken up by the multiplying *B. longum* population was being replaced via autolysis of some of the microbial cells in the soymilk. Since nitrogen is required for growth and biosynthesis of components of the bacterial cells, inadequate quantity and poor quality of free amino acids could activate bacterial proteolytic enzymes so that the amino acids might increase as a result of hydrolysis of the milk substrate (Brankovi & Baras, 2001). One of the beneficial effects of *Bifidobacteria* is the preservation of nitrogen content by preventing putrefaction thus it has been reported that the adaptation of *Bifidobacterium* species to gastrointestinal tract enhances nitrogen retention (Gibson *et al*., 1997).

The crude protein content for samples stored at ambient temperature also increased gradually from 1.76 to 2.14% and from 1.76 to 1.96% for samples stored at refrigeration temperature (Table 3). This could have been caused by increase in the production of proteins from the multiplication of microbial cells, which are mainly proteins. Amino nitrogen and crude protein were not significantly different at ambient and refrigeration temperatures of storage ($p > 0.05$).

3.7 Total reducing sugar and carbohydrate content

The total reducing sugar content of the test samples (Table 3) showed an increase by the slowly multiplying *Bifidobacteria* population in the samples from 1.28 to 3.77% for samples stored at ambient temperature and from 1.28 to 2.49% for samples stored at refrigeration temperature by the 7th day of storage. This increase in reducing sugar content of the test samples was possibly due to the production of reducing sugars via hydrolysis of the predominant α – galactosyl oligosaccharides including raffinose and stachyose present in the soymilk (Tsangalis *et al*., 2002). These sugars have been found to be satisfactory fermentable substrate for the growth of bifidobacteria in soymilk (Garro *et al*., 1999; Tsangalis *et al.,* 2002). This is because it has been shown from experimental studies that *Bifidobacterium* species produce intracellular α – galactosidase required to metabolize these sugars (Tsangalis *et al*., 2002). The subsequent reduction in the sugar content of the test samples is suggested to be due to its utilization (Garro *et al*., 1999; Brankori & Baras, 2001). The slightly higher values observed in the refrigerated test samples were due to slower utilization.

The initial sharp reduction in carbohydrate values observed between the 1st and the 7th day of storage from 3.75 to 1.67% for samples stored at ambient temperature could be related to hydrolysis of the carbohydrate by amylolytic enzymes of the probiotic bacteria resulting in the increased reducing sugar production observed during the same period (Rani & Khetarpaul, 1999; Tsangalis *et al*., 2002) and thus starch digestibility.

4. Conclusion

It can be concluded that of the two *Bifidobacteria* species isolated from breast-fed babies, *B. longum* was found to proliferate in soymilk for 35 days. Also, the microbe exhibited antimicrobial activity against *E. coli* and *S. aureus* though to a greater degree in the former than the latter. In addition, soymilk which supported the potentially probiotic *B. longum,* has also been shown to have great potentials in the formulation of probiotic beverages.

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Table 1. Identification of human intestinal *Bifidobacterium* isolates

TESTS	ISOLATES					
	1	2	3	4	5	6
Morphology	Club shaped, Long thin rods	Club shaped, V-, and Y-shaped	Club shaped long rods	Club shaped long rods	Club shaped long rods,	Club shaped long rods,
Gram Stain	+	+		+	+	+
Catalase test	-		-			-
Sugar Fermentation	g.x 1000)le 4. soymilk samples. protein ash, moisture, fatolids 167167167167 167167167167 167167167167 167167167167 167167167167 167167167167 167167167167 167167+	W	+	+	+	+
Xylose		+	+	+	+	+
Fructose		-	-	-	-	-
Maltose	+	-	-	+	+	+
Rhamnose	-	-	-	-	-	-
Sorbose	+	+	ND	ND	ND	ND
Glycerol	-	ND	+	+	+	+
Erythritol	+	+	-	-	-	-
Arabinose	-	-				
Adonitol	+					
Dulcitol	-					
Starch	W					
Melizitose	+					
Salicin	-					
Probable identity of organism	B. longum	B. adolescentis	B. longum	B. longum	B. longum	B. longum

Symbol: + = Positive, - = Negative, W = Weak, ND = Not determined

Table 2. pH, TTA and Total solids of inoculated soymilk in storage (%)

Storage Period (Weeks)	pH		TTA (% Lactic acid))		Total Solids content (%)	
	Ambient Temp.	Refrigerated Temp.	Ambient Temp.	Refrigerated Temp.	Ambient Temp.	Refrigerated Temp.
0	5.72±0.12	5.72±0.12	0.17±0.02	0.17±0.02	5.16±0.21	5.16±0.21
1	4.58±0.15	5.63±0.13	0.43±0.03	0.19±0.01	4.62±0.20	4.78±0.19
2	4.51±0.18	5.62±0.12	0.45±0.02	0.20±0.04	4.26±0.22	4.60±0.18
3	4.47±0.13	5.64±0.14	0.51±0.01	0.21±0.01	4.23±0.21	4.57±0.22
4	4.39±0.12	5.44±0.15	0.46±0.04	0.20±0.02	4.53±0.19	4.73±0.17
5	4.36±0.12	5.44±0.19	0.52±0.04	0.18±0.01	4.43±0.19	4.50±0.09

Table 3. Amino Nitrogen, Crude protein and Carbohydrate contents of inoculated soymilk in storage

Storage Period (Weeks)	Amino Nitrogen (mg/ml)		Crude Protein Content (%)		Total Reducing Sugar Content (mg/ml)		Carbohydrate Content (%)	
	Ambient Temp.	Refrig. Temp.	Ambient Temp.	Refrig. Temp.	Ambient Temp.	Refrig. Temp.	Ambient Temp.	Refrig. Temp.
0	0.18±0.02	0.18±0.02	1.76±0.05	1.76±0.5	1.28±0.06	1.28±0.06	3.75±0.13	3.75±0.13
1	0.22±0.01	0.22±0.01	1.84±0.11	1.76±0.07	3.77±0.19	2.49±0.20	1.67±0.09	2.23±0.10
2	0.22±0.01	0.22±0.02	1.90±0.07	1.77±0.11	0.98±0.04	1.78±0.03	1.50±0.07	1.91±0.06
3	0.22±0.02	0.20±0.02	1.95±0.02	1.79±0.08	0.98±0.02	1.25±0.02	1.43±0.10	1.19±0.05
4	0.22±0.01	0.20±0.03	2.14±0.07	2.08±0.04	0.87±0.01	1.09±0.05	1.21±0.04	1.19±0.06
5	0.22±0.02	0.22±0.01	2.14±0.09	1.96±0.02	0.75±0.02	1.09±0.02	1.21±0.06	1.24±0.07

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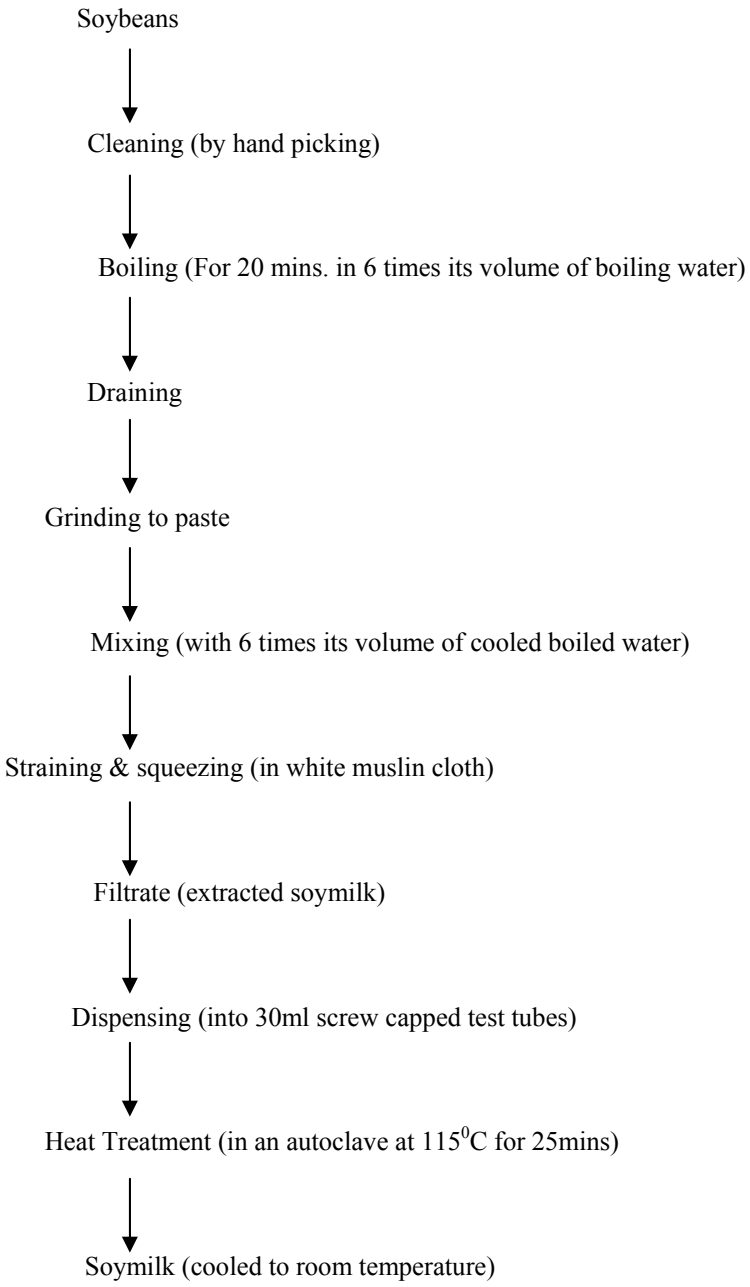

Figure 1. Flow chart for the preparation of soymilk (IITA, 1989)

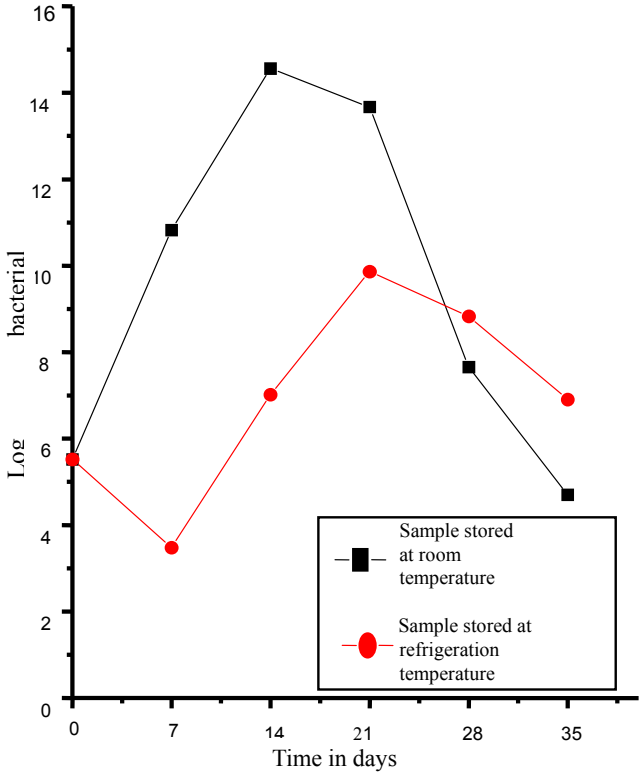

Figure 2. Viable count of B. longum in soymilk

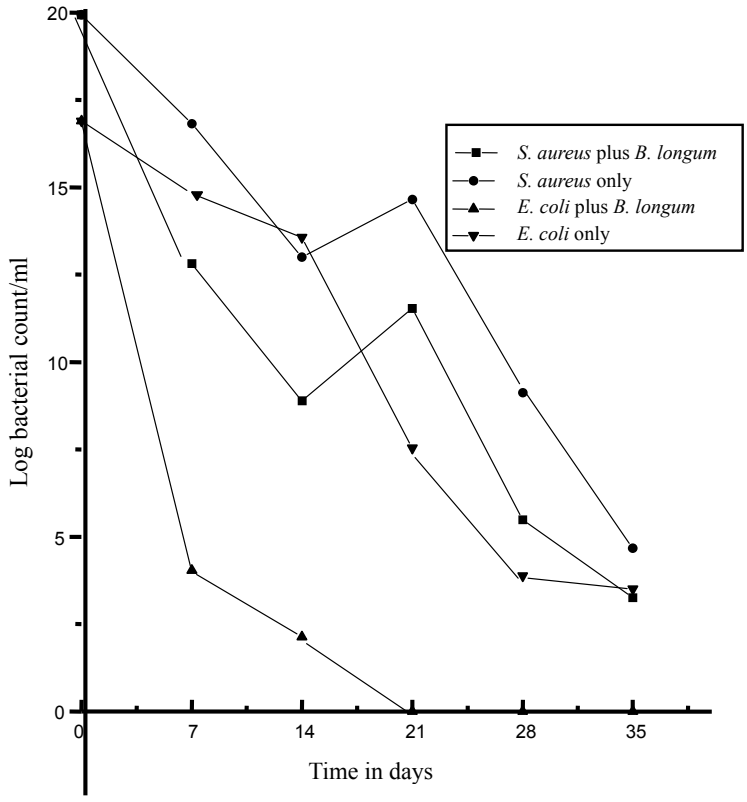

Figure 3. Antimicrobial effects of *B. longum on Escherichia coli* and *Staphylococcus aureus* in soymilk

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