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Full Length Research Paper

Microbial utilization of stachyose in soymilk yogurt production

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Conventional dairy yogurt starter cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were studied for their ability to reduce stachyose in soymilk after 8 h fermentation at 44°C. The bacteria required acclimatization and propagation in soymilk prior to inoculation of fermentation medium. Plain soymilk fermented by inocula developed from the starter cultures stored in soymilk at –10°C for 24, 168 and 360 h had 14.8, 26.7 and 31.5% reduction in stachyose concentration respectively after 8 h fermentation at 44°C.

Key words: Stachyose reduction, soymilk yogurt production.

INTRODUCTION

Soybean, (*Glycine max*) (L) Merr. is economically the most important bean in the world, providing vegetable protein for millions of people and ingredients for hundreds of chemical products (Wynstra, 1986). Traditionally lactic acid bacteria are used to prepare fermented milk products. Growth of lactic cultures in soymilk have been reported in the literature (Mital and Steinkraus, 1974; Pinthong et al., 1980.

The major carbohydrates in soybeans are sucrose, raffinose and stachyose. The main problem limiting the widespread consumption of soybeans is their objectionable beany flavour and high levels of oligosaccharides, raffinose and stachyose, which can lead to flatulence when consumed in large amounts (Cristofaro et al., 1974). Some processes have been developed to lessen the development of undesirable flavours during processing (Wilkens et al., 1967; Mustakas et al., 1969), and attempts have been made to reduce the levels of oligossachrides in soyproducts by leaching and enzyme hydrolysis (Sugimoto and Van Buren, 1970). However, both of these methods are expensive, and there is an additional risk of losing valuable protein in the process.

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An alternative approach would be the utilization of microorganisms possessing α-galactosidase activity which can hydrolyse the oligosaccharides during fermentation. This would provide simple sugars as substrate for the lactic acid bacteria as well as reduce the level of oligosaccharides present. Some researchers (Mital and Steinkraus, 1975; Pinthong et al., 1980) have reported the reduction of oligosaccharides during fermentation of soymilk using either pure or mixed culture of the following bacteria: *Lactobacillus cellobiosis, L. plantarum, L. fermentum, L. delbrueckii, L. fermenti, L. pentosaceus* and *L. bulgaricus*.

The purpose of this investigation was to determine the extent of utilization of soybean stachyose by commonly used yogurt bacteria: *L. bulgaricus* and *Streptococcus thermophilus* in mixed culture during soymilk fermentation. The principal focus is on method of starter culture preparation involving acclimatization of baceria in soymilk samples for different lengths of time (24, 168 and 360 h) and propagation in soymilk prior to inoculation of fermentation medium.

MATERIALS AND METHODS

Soybean sample

Soybeans were obtained from Edo Agricultural Development programme (ADP) in Benin City, Nigeria.

Source of cultures

Bacterial strains used were laboratory stock cultures isolated from commercial yogurt samples obtained from FAN MILK PLC, Ibadan, Nigeria. The cultures were maintained by biweekly transfers into sterile litmus milk or soymilk and held at 5°C between transfers.

Soymilk preparation

To prepare soymilk, 112 g of soybeans were soaked for 14 – 16 h in 1 L of distilled water at room temperature (28°C) in a 2.0 L beaker. The soak water was drained from the soybeans and the beans thereafter were blanched at 98°C in boiling distilled water for 20 min. The drained beans were hand washed thoroughly to remove their testa. They were then placed in a warring blender and 1.0 L of boiled distilled water at 87 – 90°C was added and then blended for 3 min. The boiled water inactivated the enzyme, lipoxygenase during blending (Wilkens et al., 1967). The resulting slurry was filtered through two layers of 50 mesh cheese cloth and approximately 990 ml of soymilk was obtained per 112 g of soybeans in 1 L of water. Flasks containing soymilk were capped with cotton plugs, covered with aluminium foil and autoclaved at 121°C for 15 min and thereafter held at 5°C until used.

Inoculum development

The stock cultures of *L. bulgaricus* and *S. thermophilus* which were first acclimatized in soymilk for different lengths of time; 24, 168 and 360 h at –10°C were employed in inoculum development. 5 ml vial of the pure culture was used to inoculate each first-set flask containing 100 ml soymilk. These subcultures were incubated for 24 h at 44°C. Second set flasks were inoculated and incubated for 12 h at 44°C. Third set flasks were also inoculated and incubated for 3 h at 44°C. In this study all inocula were sub-cultured as pure cultures up to the third-set flasks. The fourth-set flasks were inoculated with a mixed culture of the two pure isolates developed in the same manner.

Fermentation of soymilk

Three sets of fermentation units were prepared as A, B. and C. In A, B and C, the inoculum was developed for lactic acid bacteria (*S. thermophilus* and *L. bulgaricus*) acclimatized at –10°C in soymilk for 24, 168 and 360 h, respectively, and also sub-cultured in soymilk prior to inoculation. In these studies, soymilk fermentations were conducted in 500 ml capacity flasks. Aseptic conditions were maintained in the fermentation units by use of cotton plugs covered with cheese cloth and aluminium foils. 5 L of soymilk was autoclaved in a 6 L flask fitted with cotton plug and covered with cheese cloth and aluminium foil. A 5% volume of total inoculum consisting of a mix from each pure culture flask was added to 5 L of sterile soymilk in a 6 L Erlenmeyer flask. The entire content of the 6 L flask was mixed thoroughly for approximately 2 min. Sterile 50 ml flasks were filled aseptically and placed in an incubator at 44°C. Sampling was carried out every 1 h during soymilk fermentation. One flask was removed at each sampling time and served as the sample for analyses. Fermentation lasted for 8 h.

Physico-chemical analyses

All pH measurements were taken with a digital model 7065 pH meter. The per cent lactic acid produced in soymilk yogurt fermentation was expressed as the volume (ml) of 0.1 N NaOH added per ml of sample multiplied by 100 (AOAC, 1984).

Total solids and moisture

Total solids and moisture content of samples were determined as described by Osborne and Voogt (1978) based on the principle of drying to constant weight. The ash content of each sample was determined by dry-ashing in a muffle furnace at 550°C as described by Pearson (1976). Fat content was determined by AOAC (1970) procedure using the soxhlet extraction method. Crude protein was estimated by determination of the total nitrogen following the kjeldahl method (AOAC, 1970) with conversion factor of 6.25.

Determination of stachyose concentration

Stachyose concentration was measured by High Performance Liquid Chromatography (HPLC) technique. Carbohydrate was extracted from the soymilk and prepared for injection into the HPLC using the method developed for bovine milk by Kwak and Jeon (1986). 10 ml of soymilk or yogurt mix sample were mixed with 15 ml of 2-propanol and the resulting mixture centrifuged. The supernatant was filtered in a 5.0 cm diameter Buchner filter using Whatman No. 50 filter paper with a General Electric GEL 56110 vacuum pump. The filtrate was passed through Waters C–18 SEP-PAK filter cartridges to remove residual proteins, liquids and chromophores. After prewetting and sample addition, 20 µl of the filtrate eluted from the SEP-PAK catridges were injected directly into the HPLC.

The HPLC used was Varian model 5,000 Liquid Chromatograph with LC–NH_2 (5 micron) column (Sulpeco, U.S.A). Column dimensions were 250 cm by 4.6 cm internal diameter and the temperature of operation was 30°C. The mobile phase was acetonitrile/water with a flow rate 1.0 ml/min. The detector was a Waters Differential Refractometer model R401. The polarity was set to positive. Peaks were recorded using a Varian model 9176 strip chart recorder set at 1.0 cm/min chart speed and 100 mV span. A 70:30 acetonitrile to water mixture was used on the sulpeco column for the separation of the sugars present in the sample. Peak identification was based upon a documentation of the retention times of standard solutions of the pure sugars sampled on the HPLC under conditions identical to those used for the test samples. Quantification was carried out by peak area comparisons of sample and standards of known sugar concentrations.

Microbiological analysis

The viable cell count of lactic acid bacteria (*S. thermophilus* and *L. bulgaricus*) were estimated using the standard plate count method (Collins and Lyne, 1984).

RESULTS AND DISCUSSION

The soymilk used in these experiments contained 3.2±0.1% protein, 1.8±0.03% fat, 0.46±0.01% ash, 2.10 ± 0.01% carbohydrate (by difference) with 7.56±0.03% total solids. Mean pH, titratable acidity (% lactic acid) and stachyose concentration were 6.4 ± 0.02, 0.17 ± 0.01% and 328 ± 2 mg/100ml, respectively.

Stachyose reduction by mixed culture of *L. bulgaricus* and *S. thermophilus* is presented in Figure 1. In Figure 1a is shown the results of study A in which the inoculum acclimatized in soymilk at –10°C for 24 h. Stachyose decreased from 330 to 281 mg/100 ml, corresponding to 14.8% reduction. The initial viable cell count of lactic acid

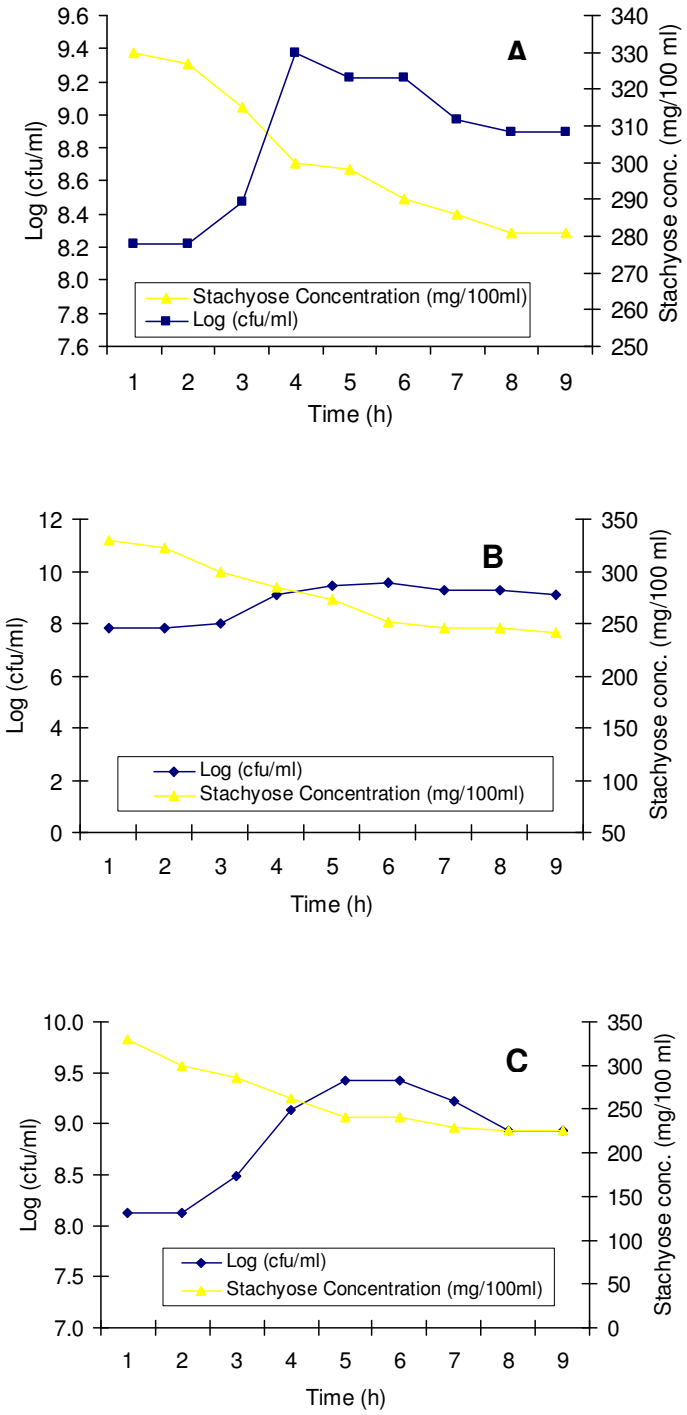

Figure 1. Stachyose utilisation in soymilk fermentation *by a mixed culture of L. bulgaricus* and *S. thermophilus*. Soymilk Fermentation with inoculum acclimatized in soymilk at –10°C for 24 h (study **A**), 168 h (study **B**) and 360 h (study **C**).

bacteria (*L. bulgaricus* and *S. thermophilus)* in this study was 1.67 x 10^8 cfu/ml. This value increased to 2.33 x 10^9 cfu/ml after 3 h and decreased to 8.0 x 10^8 CFU/ml after 8 h at 44°C. When the inoculum was acclimatized in soymilk at –10°C for 168 h (Figure 1b), stachyose decreased from 330 to 242 mg/100ml in 8 h of fermentation at 44°C (26.7% reduction). An excellent growth of lactic acid bacteria was observed in this study; the initial viable cell count of 6.67 x 10^7 cfu/ml increased steadily to 3.68 x 10^9 cfu/ml by 5 h but decreased to a final value of 1.33 x 10^9 cfu/ml at 8 h. In Figure 1c in which the organisms were acclimatized in soymilk for 360 h, stachyose concentration was found to decrease from 330 to 226 mg/100ml after 8 h of fermentation (31.5% reduction). Here, lactic acid bacteria population increased from 1.33 x 10^9 to 2.67 x 10^9 cfu/ml by the fifth hour but decreased to 8.53 x 10^8 cfu/ml at the end of the fermentation. Acclimatization time played significant role in stachyose reduction in soymilk fermentation.

When the bacteria were acclimatized in soymilk for 168 and 360 h, 26.7% and 31.5% reduction in stachyose, respectively, were observed. These values are considerably higher than the 16% reduction in stachyose recorded for *S. thermophilus* and *L. fermenti* by Pinthong et al. (1980). The growth of lactic acid bacteria in these experiments were also higher than those reported by Mital and Steinkraus (1974). Finally, when the common yogurt bacteria (*L. bulgaricus* and *S. thermophilus*) is acclimatized in soymilk at –10°C for 360 h and subjected to various subculturing procedures prior to inoculation of fermentation medium, reductionin the flatulent sugar (stachyose) in soymilk is observed after 8 h of fermentation at 44°C. Acclimatization of lactic acid bacteria in soymilk at –10°C for 360 h may have caused an enzyme induction which facilitated the reduction of the oligosaccharide stachyose by the bacteria.

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