

A Research Note

Destruction of Indigenous *Bacillus* Spores in Soymilk by Heat¹

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ABSTRACT

Boiling of soymilk in preparation for soy yogurt production does not destroy the indigenous *Bacillus* spores which may affect the fermentation process. Soymilk (2.2×10^3 CFU/ml) boiled for 1 min in a Microwave had 33 CFU/ml, steamed for 20 min at 110°C had 25 CFU/ml, and autoclaved at 121°C for 15 min had no detectable organisms ($<1 \times 10$). Soy yogurt made from these heat-treated soymilks had 4.2×10^6 *Bacillus* spores/ml, 3.4×10^4 *Bacillus* spores ml, and no detectable spores, respectively. We recommend autoclaving (121°C, 15 min) soymilk before performing research work on microbiology and biochemistry of soy yogurt fermentation.

Soymilk, an inexpensive protein source, is an important substrate for the production of soybean curd and soy yogurt-like products. Little discussion in the literature has been given to the microbial loads of soymilk after conventional heat treatment (2). Several different exposure times and temperatures of heat treatment have been used with soymilk (2,3,4,5,6). None of the studies has given any indication of the absence of detectable microorganisms of the soymilk after heat treatment. Soybean is a potential carrier of *Bacillus* spores which may survive mild treatment and may affect subsequent soymilk fermentation.

The purpose of this investigation was to evaluate three heat treatments to ascertain the microbial loads of soymilk for soy yogurt fermentation.

MATERIALS AND METHODS

Soymilk preparation

Soy beans (Cultivar Williams 82, 55.5 g) were first soaked for 14-16 h in 500 ml of distilled water at room temperature. The soaked beans were then blended in a Waring blender for 3 min. The resultant slurry was filtered through 2 layers of 50 mesh cheese cloth. Approximately 490 ml of soymilk were obtained from 55.5 g of soybeans.

Heat treatments

Two hundred milliliters of soymilk were placed in a 1.0 L beaker and covered loosely with Glad brand microwave wrap.

The soymilk was heated in a Sampo model 15041 microwave oven (full power) for 60 s once the boiling point had been reached. The wrap sealed the beaker rim instantly upon removal of the beaker from the oven due to vacuum effect. The beaker containing the boiled soymilk was stored at 4°C until used (overnight). For steam heating, the soymilk was placed in an autoclave set at 110°C for 20 min and for autoclaving, the soy milk was placed in the autoclave for 15 min at 121°C. Viable cell counts were made using standard plate count methods and standard plate count agar before and after these heat treatments. The plates were incubated for 48 h at 35°C before counting of colonies.

The heat-treated milk, after cooled to 44°C was inoculated with a 5% inoculum containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* at a ratio of 5 cocci to 1 rod. The cell density of the inoculated soymilk was ca. 5×10^6 colony forming units (CFU)/ml. The inoculated soymilk was placed in a 44°C incubator until the soymilk reached pH 4.5 (ca. 3 h) and fermented to become a soy yogurt product (1).

RESULTS AND DISCUSSION

The viable cell count of soymilk before heat treatment was 2.2×10^3 (CFU)/ml. Immediately after heat treatment the counts were 33 CFU/ml for microwave treated soymilk, 25 CFU/ml for 110°C treated soymilk, and $<1 \times 10^1$ for 121°C treated soymilk, indicating that only autoclaving at 121°C destroyed indigenous *Bacillus* of soymilk. The surviving organisms were determined to be *Bacillus* by observing colony morphology on the agar plates as well as stained cells (methylene blue) under oil immersion microscopy. Typical *Bacillus* cells and spores were observed.

After these heat-treated soymilks were made into soy yogurt by the inoculation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, viable cell counts were made. Since at 35°C incubation temperature *L. bulgaricus* and *S. thermophilus* will not grow in standard plate count agar, all counts were considered *Bacillus* counts. Soy yogurt made from microwave treated soymilk had 4.3×10^6 *Bacillus*/ml and soy yogurt made from 110°C treated soymilk had 3.4×10^4 *Bacillus*/ml. However, autoclaved soymilk

(121°C for 15 min) resulted in no *Bacillus* spores after fermentation of the soymilk.

We recommend that soymilk be sterilized by autoclaving for 15 min at 121°C before use as substrate for yogurt fermentation experiments to avoid interference of biochemical activities of indigenous *Bacillus*. The possible effects of indigenous *Bacillus* on the fermentation of soy yogurt is a subject of further investigation.

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