PAPER

MICROBIOLOGICAL QUALITY OF VEGETABLE PROTEINS DURING THE PREPARATION OF A MEAT ANALOG

QUALITÀ MICROBIOLOGICA DELLE PROTEINE DI ORIGINE VEGETALE DURANTE LA PREPARAZIONE DI UN PRODOTTO ANALOGO DELLA CARNE

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ABSTRACT

A canned vegetable protein product (or meat analog) consisting of wheat gluten, textured soy protein, hydrogenated vegetable fat and seasonings was analyzed to study the characteristics of the raw materials, estimate the microbial bioburden of the canned product prior to retorting and evaluate processing stages critical in the control of microbial growth. Two hundred and eighty-eight lots of this product were analyzed within a 24-month period. The highest aerobic mesophilic counts were

RIASSUNTO

Gli obiettivi di questo lavoro erano quelli di studiare le caratteristiche delle materie prime (proteine di soia strutturate e glutine di frumento) di un prodotto in scatola di origine vegetale, analogo della carne, valutare la carica microbica del prodotto inscatolato prima del processo di sterilizzazione e valutare i punti critici di processo, per il controllo della crescita microbica. Lotti (288) di questo prodotto sono stati analizzati durante un periodo di 24 mesi. I più alti livelli di flora batterica aerobica mesofi-

- Key words: Bioburden, canned food, meat analog, vegetable protein product -

4.96 log CFU/g for soy protein raw material, 6.76 log CFU/g for ground soy protein and 7.15 log CFU/g for canned meat analog before retorting at 121°C. The high bioburden level of textured soy protein and exposure of this raw material to conditions that promoted growth during processing caused an increase in the pre-processing bioburden. However, the sterility tests of the final product demonstrated the efficacy of the retorting process.

la riscontrati sono stati 4,96 log UFC/g per le proteine di soia grezze, 6,76 log UFC/g per la proteina di soia macinata e 7,15 log UFC/g per il prodotto analogo della carne inscatolato prima del trattamento di sterilizzazione a 121°C. L'alta carica microbica della proteina di soia e l'esposizione di questa materia prima a condizioni che hanno promosso la crescita microbica durante il processo di lavorazione hanno causato un incremento della carica microbica prima della sterilizzazione. Comunque, i test di sterilità condotti sul prodotto finito hanno dimostrato l'efficacia del processo di sterilizzazione.

INTRODUCTION

Vegetarianism is receiving more attention than ever before from both the general public and the food industry. There is a strong and growing demand for food that is natural, convenient and contains few additives. Although there has been a marked increase in the sale of vegetarian food, the safety of these products should be fully evaluated (MESSINA and MESSINA, 1997). In the United States, the "meat analog (and substitute)" category has achieved double-digit growth annually for the last five years (RAJARAM and SABATÉ, 2000). Scientific evidence points to a correlation between a vegetarian diet and reduced risk of such chronic diseases as obesity, coronary disease, hypertension, diabetes, and some types of cancer. Historically, vegetarian diets have met nutritional needs in many cultures, but other reasons for being vegetarian include religious beliefs, economic status, environmental and ecological concerns, and personal health.

Meat analogs are products processed from vegetable protein to resemble meat in texture and flavor. Meat analog products can be obtained via the following procedures: (i) spinning isolated soy protein into a "tow" of fibers, followed by shaping and flavoring to make meat-like products; (ii) processing formulated soy flour through an extruder into different sizes and shapes to give chewable products with the desired flavor, and (iii) by using heat and pressure during continuous extrusion of soy protein formula of small chunks which, when hydrated, present a chewable texture and a taste similar to meat. The principal advantage of this third method is the use of low cost soy flour in the preparation rather than the more expensive isolated protein (SMITH and CIRCLE, 1972).

Meat analogs are made from natural sources, such as soybeans, peanuts, wheat, and corn. The biological quality of the protein in the vegetable protein product should be at least 80 percent casein, which is determined by using the Protein Efficiency Ratio (PER) scale. The meat analog should contain at least 18 percent protein of the hydrated weight (w/w) after preparation (USDA, 1991).

Wheat gluten alone, when measured against the standard casein reference

(PER = 2.5) in rat bioassays, rates rather low on the PER scale, from 0.7 to 1.0 or 28 to 40% of the casein value. Vegetable proteins, in general, rate low on the PER scale because they frequently lack one or more essential amino acids. Blends of different vegetable proteins often result in higher PER values. For a wheat gluten/soy protein ratio of 30:70, a PER value of 2.4 is achieved, or roughly three times the rating of wheat gluten alone (AAOCC, 1964).

The canned vegetable protein product in this study consisted of wheat gluten, textured soy protein, hydrogenated vegetable fat, garlic, onion, salt, meat flavoring, caramel color and seasonings; it was processed to resemble ground meat in appearance and flavor. It is a ready-to-eat food, readily available in Brazilian supermarkets and is used in many vegetarian recipes. It is also used as a protein supplement, especially for children and adult lunch programs, at public institutions. The preservation of this canned product is assured by controlling the sanitary quality of the raw materials and the extent of heat processing.

The objectives of this paper were to study the characteristics of the raw materials (textured soy protein and wheat gluten), estimate the microbial bioburden of the canned product prior to retorting and to identify the critical processing stages to control the microbial growth.

MATERIALS AND METHODS

Meat analog manufacture

The product was prepared in a vegetable protein pilot plant (Superbom Food Industry, SP, BR), following a procedure illustrated in the flow diagram shown in Fig. 1. The raw materials (Table 1), in the proportion of 12% textured soy protein (53% protein; 1% fat; 6% moisture; 12.7 mm particle size) and 15% wheat gluten (77% protein; 0.7% fat; 1% ash; 7.5% moisture) were mixed in amounts necessary to prepare one batch of the final vegetable protein product. A production lot of approximately 200 kg corresponded to 250 metallic cans each containing 850 g of the final product. The textured soy protein was always acquired from the same supplier (Bunge Alimentos, SC, BR), as was the wheat gluten (Alvol, Mendoza, Argentina). The chemical characteristics of the raw material were analyzed by a nationally certified laboratory (Adolfo Lutz Laboratory) following the AOAC (1996).

Soy preparation

The textured soy protein was placed in a stainless steel tank (Quiminox, SP, Br) and uniformly hydrated with water (1:3 wt/wt). The mixture was maintained at 70°C for 20 min using steam provided from an open coil (perforated underside) located at the bottom of the tank. Propellers installed in the center of the tank stirred the mixture constantly. The hydrated soy protein was transferred to a basket (400 nylon mesh) attached to a bowl centrifuge (1.0 m diameter; Westfalia Separator, SP, Br) and was washed by continuous jets of water (3.0 m^3/h, at room temperature 25°-30°C) introduced in the center of the product mass, while being centrifuged at 1,200 rpm for 15 min. After washing, the soy protein was centrifuged at 1,200 rpm for an additional 20 min, to attain a mass moisture of around 60% wt/wt. Washing and drying of the soy protein was needed to reduce the natural off-flavor of soy in the final product. Soy protein was minced into particles of an average size of 1.5 cm with a rotary disc meat cutter (IbrasmaK, SP, Br) with knives (25 cm in diameter spaced 3.5 cm in the center). The ground soy protein was held for no longer than two hours before mixing with other ingredients at 45°C for 15 min.

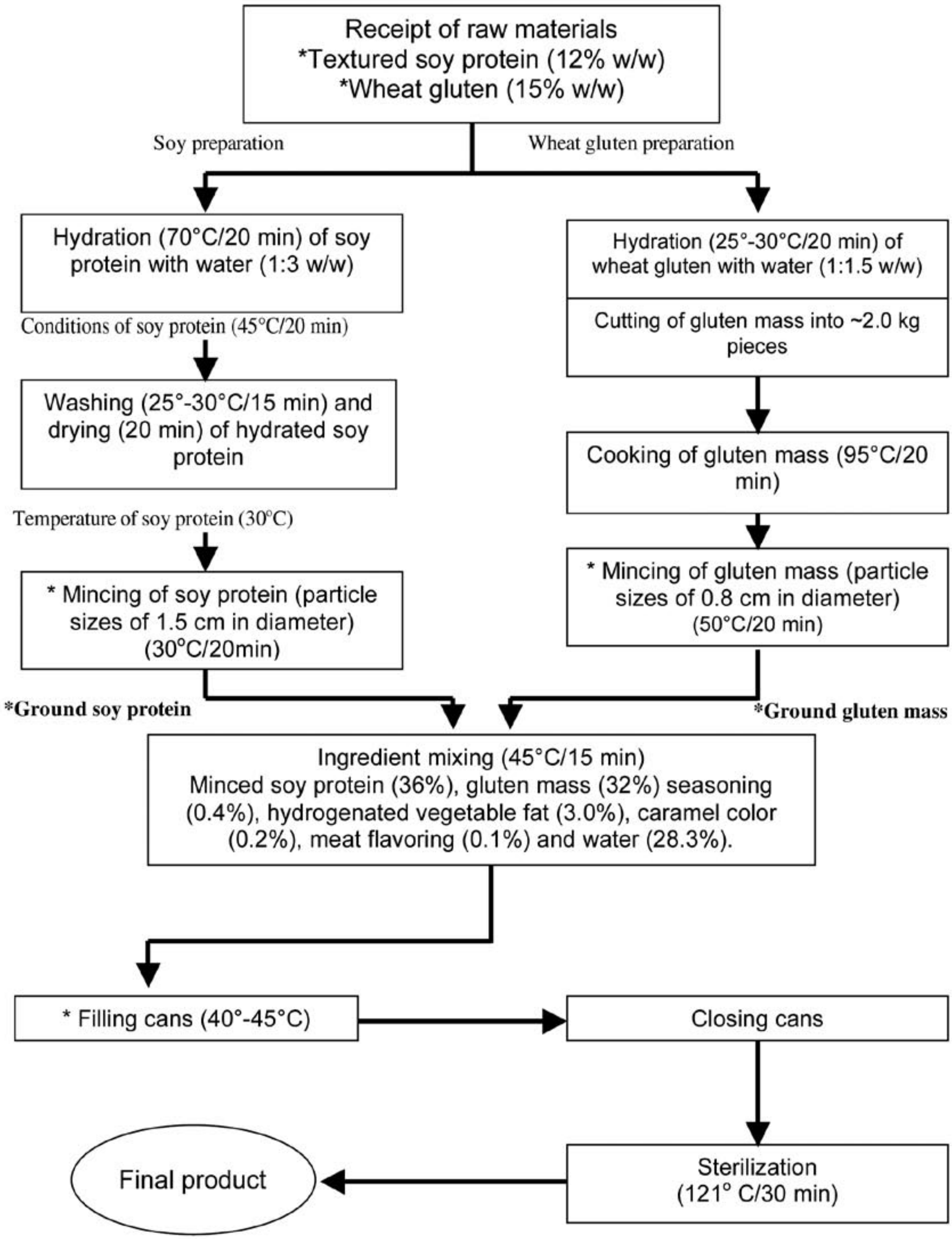

Fig. 1 - Flow diagram of vegetable protein production (*sampling points).

Table 1 - Chemical characteristics of the raw materials.

	Textured soy protein											
Year 2001	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Protein (N x 6.25) (dry basis)	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00
Fat (%)	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00
Carbohydrates (%)	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00
Fiber (%)	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00
Ash (%)	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00
Moisture (%)	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00
Year 2002												
Protein (N x 6.25) (dry basis)	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00
Fat (%)	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00
Carbohydrates (%)	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00
Fiber (%)	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00
Ash (%)	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00
Moisture (%)	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00

	Wheat gluten											
Year 2001	January	February	March	April	May	June	July	August	September	October	November	December
Protein (N x 5.7% (dry basis)	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00
Fat (%)	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00
Ash (%)	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Moisture (%)	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00
Year 2002												
Protein (N x 5.7% (dry basis)	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00
Fat (%)	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00
Ash (%)	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Moisture (%)	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00

CI = confidence interval (n = 12; $p<0.05$).

Gluten preparation

Wheat gluten was processed into a homogenous hydrated viscous mass with water (1:1.5 wt/wt) in a planetary blender (Usi Ram, SP, Br) at 80 rpm for 20 min, at a temperature of 25°-30°C. The gluten mass was then cut into pieces (about 2.0 kg/each) in a rotary disc meat cutter (IbrasmaK, SP, Br), and cooked in a water bath at 95°C for 20 min in the stainless steel tank (Quiminox, SP, Br), forming a gelatinous material stabilized by the denatured protein. The large gel pieces were cut into particles of 0.8 cm in diameter in the rotary disc cutter (IbrasmaK, SP, Br).

Mixture of ingredients and other steps

The minced soy protein (36%) and gluten mass (32%) were mixed with seasonings (0.4%), hydrogenated vegetable fat (3.0%), liquid caramel color (0.2%), meat flavors (0.1%) and water (28.3%) in a blender (Sigma-blade; 50 rpm; 250 L capacity; Usi Ram, SP, Br), at 45°C for 15 min to achieve the appearance of a moist dough with the light brown color characteristic of the meat analog product. Each cylindrical metal can (99x118 mm; with an inner facing of epoxiphenolic sanitary varnish and a lid for sealing) was filled with 850 g of the final product (kept at 40°-45°C) with an automatic filler (Welbba, SP, Br). The cans were placed in a mechanical vacuum-sealing machine, and the sealing process was completed in a vacuum of approximately 70 kPa. After passing through a warm (45°-50°C) water shower to remove any product adhering to the surface, the cans were distributed among three autoclave trucks (316 cans per truck) for sterilization (set at 121°C for 30 min, with total processing time of 88 min from initial ramping up to cool down).

Microbiological analyses

Microbiological analyses were performed on 288 lots covering two successive years of production. The number of viable microorganisms prior to sterilization was determined for each lot, and included analysis of the raw materials, the ground soy protein, the gluten mass, the ingredient mixture and the final canned meat analog product prior to retorting. Eight hundred and sixty-four samples were collected; sampling occurred during 4 production days per month with 9 samples being taken at different times throughout the production day. The samples (200-250 g) were aseptically collected in sterile bags in triplicate from every stage, as proposed by ANVISA (2001). Every effort was made to insure that the samples of the raw material were representative of all lots of the manufactured products.

Twenty-five grams from each sample was transferred into a sterile Stomacher bag (16.0x26.0 cm) and mixed with 225 mL of 0.1% (w/v) peptone water, homogenized in a Stomacher (Tekmar, Model 4001, Cincinnati, Ohio, USA) for 1 min. Serial decimal dilutions of 0.1% peptone water were prepared, and samples of appropriate dilutions were poured in duplicate on plate count agar ("PCA", Difco Laboratories, Sparks, MD, USA) plates for mesophilic (37°±2°C for 48h) and thermophilic (60°±2°C) counts. Dilutions were also plated on potato dextrose agar ("PDA", Difco) plates, acidified with sterile 10% tartaric acid (pH = 3.5) for yeast and mold (25°C for 72h) counts. The colony forming unit counts (CFU/g) were performed on plates of up to 200 colonies. Reinforced clostridial medium ("RCM", Difco) incubated at 35°±0.1°C for 72h in anaerobic jars (Gas-Pack System, Cockeysville, MD, USA) were used to measure anaerobic counts. For spore enumeration, samples were heated to 80°±0.2°C for 10 min prior to being poured. For total coliforms, each sample dilution was inoculated in triplicate into lauryl sulfate tryptose ("LST", Difco) tubes, which were incubated at 35°±1°C and the results are reported as the most probable number (MPN) of coliform bacteria per gram (APHA, 1992). The positive LST tubes were checked (i) for coliform bacteria by subculture and put into brilliant green bile ("BGB", Difco) broth tubes and incubated at 35°±0.5°C for 48±2 h; and (ii) for fecal coliform bacteria by subculture into *E. coli* ("EC", Difco) broth tubes, and incubated for 24±2 h at 45.5°±0.2°C in a controlled water bath.

Analysis of final retorted product

The final composition of the canned product was analyzed in accordance with AOAC (1996). The final retorted product was analyzed by the commercial sterility test by incubating 20 cans of each lot at 37°±2°C for 10 days. Cans were also examined for abnormal conditions such as leakage, swelling, and odor. The vacuum and pH were measured, and microscopic examinations were performed.

Microbiological tests of process water

The central industrial reservoir receives potable municipal water. The tank walls were sanitized with sodium hypochlorite (100 ppm total chlorine) biannually during mechanical cleaning. Water used in processing vegetable protein was treated with cartridge filters (1,000 L capacity, Acqua Domini, SP, Br) consisting of four layers (active charcoal with salts of colloidal silver, quartz, and dolomite). The water was tested according to Brazilian state and federal regulations and followed the methodology of APHA (1995). The free chlorine content in the processing water was monitored daily and maintained at 0.5-1.0 ppm. The analyses of standard aerobic mesophilic and coliform counts were conducted twice each week.

Microbiological testing of equipment, facilities and worker's hands

The analyses of samples from the equipment, facilities and worker's hands in the processing plant were performed according to Brazilian state and federal regulations and followed the methodology of APHA (1992).

Approximately 100 cm^2 per area was swabbed using a rayon-tipped applicator. Samples of surfaces of worker's hands were collected by the "swab contact method" and subjected to the same microbiological analyses as equipment swab samples. Before the first lot and at the end of the production day, the following analyses were performed after cleaning and sanitizing of the equipment: total count of mesophilic and coliform bacteria, *Staphylococcus aureus* (coagulase positive), yeasts and molds. Cleaning and sanitizing the equipment and facilities consisted of an initial rinse with potable water (45°C) to remove gross contamination, followed by washing with a 2% v/v alkaline (62% NaOH) non-ionic detergent solution (Quimistrol SU 186, Lever Industrial, SP, Br) at 45°C for 10 min and rinsing again with potable water. Next, the equipment and facilities were sanitized with a solution (1% v/v) of sodium hypochlorite (Divosan Sol L, pH 11.5; Lever Industrial SP, Br) at 5 ppm residual chorine for 5 min, followed by a water rinse.

Statistical analysis

All microbiological counts were converted to decimal logarithms for statistical analysis. Data were analyzed with the SGWIN program (Statgraphics Plus for Windows version 1.4 – Statistical graphics Corporation, 1995). The mean, standard deviation, and range were calculated for every n = 12 samples collected. Differences between means were detected by a one-way analysis of variance and are expressed as confidence intervals (CI). Probabilities of ≤0.05 were considered significant.

RESULTS AND DISCUSSION

The canned meat analog (pH = 6.58±0.04) had a protein content greater than 18% which is the minimum required for vegetable protein products (SMITH and CIRCLE, 1972) in accordance with the following percentages of the product: protein (dry basis; Nx6.25%=20.69±0.80%), fat (1.92±0.37%), carbohydrates (2.90±0.34%), moisture (69.48±0.55%), fiber (1.47±0.18%), iron (1.65±0.12 mg/100 g) and phosphorous (63.30 mg/100 g), totaling 127.00±1.49 calories per 100 g of product. The high-protein and moisture content of the canned product make it a highly favorable medium for microorganisms before it is processed. It is essential to maintain sanitary conditions of the raw materials during the processing stages as this contributes to the final microbial bioburden of the product.

The microbiological characteristics for the water used in the different steps of preparation met the requirements of APHA (1995). The standard plate counts were less than 2 $\log_{10}$ CFU/mL of water analyzed and no coliforms were detected. Samples from the equipment and workers in the processing plant were negative for *S. aureus* and for yeasts and molds. In addition, coliforms and mesophilic groups were present at less than 2.0 $\log_{10}$ CFU/cm^2.

The samples of each raw material presented a range of standard counts in plates from a minimum of 0.50 $\log_{10}$ CFU/g to a maximum of 1.50 $\log_{10}$ CFU/g for wheat gluten; from a minimum of 4.61 $\log_{10}$ CFU/g to a maximum 4.96 $\log_{10}$ CFU/g for textured soy protein. Yeast and mold counts ranged from 0.42 to 1.75 $\log_{10}$ CFU/g for wheat gluten to an average of 2.0 $\log_{10}$ CFU /g for textured soy protein. The absence of coliforms and *S. aureus* in 1.0 g of analyzed sample, and of *Salmonella* spp. in 25 g of sample was certified in every raw material (Table 2). The high microbial populations present in the soy protein raw material shows a potential risk that must be controlled during processing.

KELLER *et al.* (2002) evaluated the efficacy of sanitation and cleaning methods in a small apple cider mill by surface swab methods as well as the microbiological characteristics of the incoming raw ingredients and the final product. They observed that, in general, the microbiological quality of the final product reflects the microbial load of the raw ingredients.

Soy preparation

After hydration, washing and grinding of the textured soy protein (Fig. 1), the average standard count increased by as much as 2 $\log_{10}$ cycles (Table 3) with respect to the microbial populations of the raw material. The highest total mesophilic aerobic count was observed in November 2002 (6.76±0.20), while the highest total mesophilic anaerobic count was observed in April 2001 (5.65±0.11 $\log_{10}$ CFU/g). The highest yeast and mold count was observed in September 2001 (3.64±0.16 $\log_{10}$ CFU/g). The total thermophilic anaerobic and aerobic counts were generally lower and ranged from 1 to 2 $\log_{10}$ CFU/g. Despite the apparent absence of coliforms within the soy protein raw material, an average population of greater than 3 $\log_{10}$ MPN/g for coliforms and fecal coliforms was observed in the ground soy protein. This increase may have resulted from the favorable temperature and time conditions present during the hydration and washing stages prior to retorting and /or the transfer of microorganisms from the equipment to the ground product.

At the end of hydration (70°C for 20 min) the temperature of the soy protein was approximately 45°C, an ideal

Table 2 - Microbiological characteristics of the raw materials. The counts are expressed in decimal logarithms.

	Textured soy protein(log CFU/g)											
Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Standard plate count	4.61±0.20	4.92±0.23	4.92±0.23	4.94±0.28	4.92±0.23	4.90±0.21	4.94±0.28	4.96±0.25	4.92±0.23	4.69±0.27	4.90±0.21	4.92±0.23
*Yeasts and Molds (< 3.00)	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
*Coliforms (< 2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Eschrichia coli* (< 1.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**S. aureus* (positive coagulase) (<2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Salmonella* sp. (25 g sample)	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
Year 2002												
Standard plate count	4.61±0.20	4.92±0.23	4.92±0.23	4.94±0.28	4.92±0.23	4.90±0.21	4.94±0.28	4.96±0.25	4.92±0.23	4.69±0.27	4.90±0.21	4.92±0.23
*Yeasts and Molds (< 3.00)	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
*Coliforms (< 2.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Escherichia coli* (< 1.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**S. aureus* (positive coagulase) (<2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Salmonella* sp. (25 g sample)	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent

	Wheat gluten (log CFU/g)											
Year 2001	January	February	March	April	May	June	July	August	September	October	November	December
Standard plate count	0.58±0.38	0.67±0.44	0.67±0.28	1.00±0.42	1.00±0.42	1.50±0.30	1.25±0.35	0.58+ 0.29	1.42±0.29	0.58±0.29	0.58±0.29	0.75±0.26
*Yeasts and Molds (< 3.00)	1.67±0.00	1.25±0.00	1.25±0.00	1.75±0.00	1.75±0.00	1.50±0.00	1.50±0.00	1.58±0.00	1.50±0.00	0.83±0.00	1.00±0.00	1.50±0.00
*Coliforms (< 2.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Escherichia coli* (< 1.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**S. aureus* (positive coagulase) (<2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Salmonella* sp. (25 g sample)	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
Year 2002												
Standard plate count	0.50±0.30	0.92±0.45	0.67±0.28	0.92±0.38	1.00±0.00	1.50±0.30	1.00±0.00	0.50+ 0.00	0.75±0.26	1.25±0.00	0.92±0.45	1.00±0.42
*Yeasts and Molds (< 3.00)	0.42±0.00	0.83±0.00	0.58±0.00	0.58±0.00	1.17±0.00	1.25±0.00	0.25±0.00	0.75±0.00	1.50±0.00	1.08±0.00	1.58±0.00	1.17±0.00
*Coliforms (< 2.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Escherichia coli* (< 1.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**S. aureus* (positive coagulase) (<2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Salmonella* sp. (25 g sample)	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent

* Brasil, Resolution - ANVISA - Ministry of Health.
CI = confidence interval (n = 12; p<0.05).

temperature for coliform and fecal coliform development. Hydration of the soy protein is required for product quality and can promote microbial growth. The ground soy protein was stored for approximately 2 h prior to mixing the ingredients. This stage of production also contributed to the development of coliforms. Dried products (including the raw materials used here), in general, have been considered as one of the safest modes of food storage since the dry state does not promote microbial survival or growth. Low water activity (a_w < 0.85), among other factors (FUNG and GAILANI, 1986), prevents microbial development and may maintain populations at undetectable levels. Unfortunately, such hurdles are removed upon the addition of water for processing.

Although post-disinfection surface specifications for coliforms are not commonly available (because these values are likely to depend upon the environment, product and process) target values of <2.5 CFU/cm^2 have been suggested and are generally attainable for a range of surfaces (MOORE and GRIFFITH, 2002). It should be noted that the presence of very low concentrations (<2.0 $\log_{10}$ CFU/cm^2) of bacteria on surfaces may still be a significant risk factor for contamination thus emphasizing the importance of effective cleaning and disinfection procedures.

Wheat gluten preparation

In general, the ground gluten mass (Fig. 1) had lower viable counts (Table 4) compared to the soy protein (Table 3). The highest average counts were 3.19±0.18 (April 2002) and 1.83 $\log_{10}$ CFU/g (February and June 2001) for mesophilic aerobes and anaerobes, respectively. Less than or equal to 10 CFU/g were observed for all other counts, and fecal coliforms were never detected. It is likely that the cooking operation (95° for 20 min) required for the sliced hydrated gluten mass, helped to maintain its bioburden relatively unchanged from the raw material (Table 2).

Mixture of ingredients
and filled canned product

The product had mean total mesophilic aerobes above 6.5 $\log_{10}$ CFU/g after mixing, with a high of 7.28 $\log_{10}$ CFU/g observed in December 2001 (Table 5). Total mesophilic anaerobic counts were above 5 $\log_{10}$ CFU/g, with a high of 6.21 $\log_{10}$ CFU/g observed in April of 2002. Similar results were observed in the canned product (Table 6), since the can filling process was automated and did not contribute to the bioburden of the final product. Mesophilic sporeforming aerobic and anaerobic plate counts were generally close to or higher than 4 $\log_{10}$ CFU/g in the mixed (Table 5) and filled (Table 6) product. Thermophilic aerobic and anaerobic counts increased by about 1 $\log_{10}$ CFU/g during mixing (Table 5) relative to the counts in the ground soy protein (Table 3), most likely due to the presence of thermophilic organisms in the non-protein ingredients. Other than this exception, counts of other organisms were generally similar to those obtained in the ground soy protein.

TESSI *et al.* (2002) evaluated the microbiological quality and safety of ready-to-eat cooked foods from a centralized school kitchen in Argentina. The detection of large aerobic and *Enterobacteriaceae* counts in ready-to-eat cooked foods showed that improvements in manufacturing, cooking and delivery were needed to enhance the microbiological safety of these foods. This study highlighted the importance of the control and maintenance of appropriate temperatures during various stages of the food preparation process.

Raw materials have been reported as the primary sources of microbial contamination during the processing of various types of food (FUNG and GAILANI,

Table 3 - Microbiological characteristics of the ground soy protein. The counts are expressed in decimal logarithms (log CFU/g).

Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	5.66±1.06	5.54±1.07	4.77+ 0.22	6.63±0.12	6.69±0.41	5.96±0.57	4.68±0.62	5.61±1.14	5.45±0.70	5.07±0.30	6.04±0.40	5.87±0.49
Total mesophilic anaerobes	5.08±0.44	5.23±0.74	4.68±0.07	5.65±0.11	5,59±0,18	5.39±0.80	5,18±0,01	5.18±0.74	5.42±0.13	4.94±0.36	5.41±0.66	5.36±0.44
Total thermophilic aerobes	1.50±0.40	1.92±0.00	1.50±0.40	1.58±0.40	1,17±0,00	1.83±0.00	1.50±0.40	1.67±0.00	1.67±0.00	1.25±0.00	1.58±0.00	1.50±0.40
Total thermophilic anaerobes	1.25±0.00	1.42±0.00	1.27±0.00	1.25±0.00	1,42±0,00	1.25±0.00	1.55±0.40	1.50±0.40	1.42±0.00	1.42±0.00	1.25±0.40	1.25±0.00
Yeast and Molds	2.74±0.40	3.02±0.26	3.36±0.11	3.50±0.10	2,96±0,22	2.87±0.11	3.12±0.48	3.01±0.26	3.64±0.16	3.47±0.06	3.31±0.08	2.74±0.40
Coliforms (log MPN/g)	3.15±0.19	3.32±0.11	3.07±0.49	3.53±0.16	3,50±0,02	3.50±0.02	3.30±0.04	3.32±0.11	3.53±0.16	3.43±0.08	3.54±0.14	3.13±0.19
Fecal Coliforms (log MPN/g)	3.76±0.08	3.72±0.14	3.73±0.07	3.16±0.48	3,43±0,12	3.25±0.22	3.06±0.29	3.72±0.14	3.22±0.26	3.30±0.00	3.48±0.03	3.76±0.08
Mesophilic sporeforming aerobes	3.81±0.17	3.95±008	4.21±0.31	4.33±0.05	3,82±0,02	3.84±0.29	3.64±0.07	3.95±0.08	4.24±0.18	4.20±0.12	3.87±0.37	3.83±0.13
Mesophilic sporeforming anaerobes	4.06±0.21	3.60±0.53	4.12±0.15	4.30±0.05	4,11±0,18	4.20±0.14	3.37±0.70	3.64±0.53	4.27±0.05	4.30±0.14	4.21±0.14	4.15±0.29
Thermophilic sporeforming aerobes	0.42±0.40	0.58±0.00	0.92±0.40	0.50±0.40	0,50±0,40	0.92±0.00	0.75±0.00	0.58±0.00	0.42±0.00	0.67±0.00	0.67±0,00	0.50±0.00
Thermophilic sporeforming anaerobes	0.58±0.40	0.50±0.00	0.67±0.00	0.67±0.00	0.58±0.00	0.83±0.40	0.67±0.00	0.50±0.00	0.67±0.40	0.58±0.00	0.83±0.00	0.58±0.00
Year 2002 Groups of Microorganisms	**January Mean±CI**	**February Mean±CI**	**March Mean±CI**	**April Mean±CI**	**May Mean±CI**	**June Mean±CI**	**July Mean±CI**	**August Mean±CI**	**September Mean±CI**	**October Mean±CI**	**November Mean±CI**	**December Mean±CI**
Total mesophilic aerobes	6.13±0.63	5.83±0.62	5.91±0.45	5.79±0.79	6.27±0.55	4.88±0.57	5.94±0.36	6.47±0,12	5.99±0.61	5.77±0.74	6.76±0.20	4.95±0.62
Total mesophilic anaerobes	5.49±0.30	5.37±0.32	5.16±0.27	5.27±0.54	5.14±0.64	4.75±0.20	5.38±0.66	5.22±0.17	5.12±0.17	5.09+ 0.68	5.14+ 0.64	4.54±0.06
Total thermophilic aerobes	1.58±0.40	1.58±0.00	1.67±0.40	1.67±0.40	1.58±0.00	1.58±0.40	1.58±0.00	1.83±0.40	1.58±0.40	1.67±0,00	1.58±0.00	1.42±0.40
Total thermophilic anaerobes	1.42±0.00	1.33±0.00	1.42±0.00	1.50±0.40	1.42±0.40	1.25±0.00	1.25±0.40	1.25±0.00	1.33±0.00	1.58±0,40	1.67±0.40	1.27±0.00
Yeast and Molds	3.20±0.26	3.38±0.07	3.32±0.16	3.04±0.26	3.02±0.26	3.42±0.10	3.47±0.08	3.55±0.10	2.89±0.10	3.09±0,26	3.50±0.26	3.39±0.00
Coliforms (log MPN/g)	3.45±0.15	3.07±0.50	3.54±0.14	3.37±0.11	3.34±0.10	3.11±0.40	3.52±0.13	3.53±0.15	3.46±0.02	3.34±0,10	3.54±0.29	3.14±0.49
Fecal Coliforms (log MPN/g)	3.45±0,12	3.82±0.29	3.58±0.03	3.73±0.14	3.72±0.14	3.73±0.07	3.48±0.03	3.16±0.48	3.25±0.21	3.72±0,14	3.72±0.14	3.78±0.28
Mesophilic sporeforming aerobes	4.00±0.10	4.36±0.31	3.87±0.37	3.97±0.04	3.95±0.08	4.20±0.17	3.93±0.28	4.34±0.04	3.80±0.16	3.95±0,08	3.98±0.00	4.31±0.19
Mesophilic sporeforming anaerobes	4.16±0.08	4.34±0.10	4.24±0.12	3.74±0.63	3.71±0.68	4.12±0.15	4.16±0.14	4.41±0.37	4.25±0.14	3.65±0.53	3.76±0.56	4.19±0.15
Thermophilic sporeforming aerobes	0.50±0.00	1.00±0.00	0.50±0.00	0.67±0.00	0. 58±0.00	0.83±0.00	0.83±0.00	0.58±0.00	0.83±0,00	0.42±0.00	0.50±0.00	0.83±0.00
Thermophilic sporeforming anaerobes	0.58±0.00	0.58±0.00	0.75±0.00	0.50±0.00	0.58±0.00	0.67±0.00	0.67±0.00	0.65±0.00	0.83±0,00	0.58±0.00	0.50±0.00	0.50±0.00

CI = confidence interval (n = 12; p<0,05).
MPN = most probable number.

Table 4 - Microbiological characteristics of the ground gluten mass. The counts are expressed in decimal logarithms (log CFU/g).

Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	2.71±0.23	2.93±0.22	3.15±0.00	3.17±0.00	2.96+ 0.44	3.08±0.40	2.86±0.01	2.74±0.04	3.04±0.16	3.12±0.24	3.18±0.02	3.14±0.18
Total mesophilic anaerobes	1.50±0.40	1.83±0.00	1.25±0.00	1.67±0.00	1.67±0.00	1.83±0.00	1.67±0.40	1.50+ 0.00	1.50±0.40	1.75±0.00	1.42±0.40	1.50±0.40
Total thermophilic aerobes	0.58±0.00	0.67±0.40	0.58±0.00	0.58±0.00	0.50±0.00	0.67±0.00	0.58±0.00	0.50±0.00	0.50±0.40	0.58±0.00	0.58±0.00	0.58±0.00
Total thermophilic anaerobes	0.58±0.40	0.75±0.00	0.58±0.40	0.58±0.40	0.58±0.40	0.58±0.40	0.67±0.00	0.58±0.40	0.58±0.40	0.58±0.40	0.58±0.40	0.50±0.40
Yeast and Molds	0.92±0.40	1.00±0.00	0.92±0.40	0.92±0.40	0.92±0.40	1.00±0.00	0.92±0.40	0.92±0.40	0.92±0.40	0.83±0.00	0.92±0.40	0.92±0.40
Coliforms (log MPN/g)	0.92±0.00	0.83±0.00	0.75±0.00	0.92±0.40	0.92±0.00	0.75±0.40	0.92±0.00	0.92±0.00	0.83±0.00	0.83±0.00	0.83±0.00	0.75±0.00
Fecal Coliforms (log MPN/g)	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence
Mesophilic sporeforming aerobes	0.92±0.00	0.92±0.00	1.00±0.00	0.92±0.00	0.58±0.40	0.92±0.00	0.75±0.00	0.83±0.00	0.83±0.40	0.83±0.00	1.00±0.00	0.92±0.00
Mesophilic sporeforming anaerobes	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.00	0.83±0.00	0.83±0.00	0.58±0.00	0.92±0.00	0.83± 0.00
Thermophilic sporeforming aerobes	1.00±0.00	0.83±0.00	0.92±0.00	0.92±0.00	1.00±0.00	0.92±0.40	0.92±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	0.92±0.00
Thermophilic sporeforming anaerobes	0.83±0.00	0.33±0,40	0.75±0.00	0.75±0.00	0.58± 0.00	0.75±0.40	0.75±0.40	0.67±0.00	0.58±0.40	0.50±0.40	0.67±0.00	0.75±0.40

Year 2002 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	3.17±0.32	2.58±0.00	2.91±0.20	3.19±0.18	3.09±0.00	2.71±0.23	3.18±0.10	3.17±0.39	3.07±0.08	3.14±0.05	2.86±0.40	2.88±0.40
Total mesophilic anaerobes	1.75±0.00	1.50±0.00	1.33±0.40	1.50±0.40	1.67±0.00	1.50±0.40	1.33±0.40	1.67±0.00	1.50±0.40	1.50±0.40	1.67±0.00	1.25±0.40
Total thermophilic aerobes	0.58±0.00	0.50±0.00	0.50±0.40	0.58±0.00	0.58±0.00	0.58±0.00	0.58±0.00	0.50±0.00	0.58±0.00	0.58±0.00	0.58±0.00	0.58±0.00
Total thermophilic anaerobes	0.50±0.40	0.58±0.40	0.67± 0.40	0.58±0.40	0.67±0.00	0.58±0.40	0.42±0.40	0.58±0.40	0.58±0.40	0.58±0.40	0.42±0.40	0.83±0.00
Yeast and Molds	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.00	0.83±0.40	0.92±0.40	0.83±0.40	0.83±0.00
Coliforms (log MPN/g)	0.83±0.00	0.92±0.00	0.92±0.00	0.75±0.00	0.92±0.00	0.92±0.00	0.75±0.40	0.83±0.00	0.75±0.00	0.83±000	0.67±0.40	0.83±0.00
Fecal Coliforms (log MPN/g)	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence
Mesophilic sporeforming aerobes	0.92±0.00	0.83±0.00	0.67±0.40	0.83±0.40	0.75±0.00	0.92±0.00	0.92±0.00	0.83±0.00	1.00±0.00	1.00±0.00	0.83±0.00	0.67±0.40
Mesophilic sporeforming anaerobes	0.83±0.00	0.83±0.00	0.92±0.00	0.83±0.00	0.92±0.00	0.92±0.00	0.83±0.00	0.92±0.00	0.92±000	0.92±0.00	0.75±0.00	0.75±0.00
Thermophilic sporeforming aerobes	0.75±0.00	0.83±0.00	0.92±0.00	0.75±0.00	0.92±0.00	1.00±0.00	1.00±0.00	1.00±0.00	0.92±0.00	0.92±0.00	1.00±0.00	0.67±0.00
Thermophilic sporeforming anaerobes	0.67±0.00	0.75±0.40	0.67±0.00	0.58±0.40	0.83±0.00	0.83±0.00	0.58±0.40	0.67±0.40	0.67±0.00	0.67±0.40	0.58±0.00	0.67±0.00

CI = confidence interval (n = 12; p<0.05).
MPN = most probable number.

Table 5 - Microbiological characteristics of the mixture of ingredients. The counts are expressed in decimal logarithms (log CFU/g).

Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	6.85±0.42	6.89±0.32	6.52±0.28	7.03±0.54	6.78±0.07	7.15±0.12	7.09±0.20	7.06±0.06	7.00±0.02	7.11±0.08	6.86±0.33	7.28±0.12
Total mesophilic anaerobes	5.52±0.20	5.25±0.64	5.88±0.64	5.74±0.77	5.42±0.68	6.03±0.14	5.11±0.24	5.83+ 0.66	5.58±0.68	6.03±0.02	6.13±0.36	6.02±0.64
Total thermophilic aerobes	2.62±0.91	2.75±0.55	2.94±0.37	2.77±0.58	2.93±0.37	2.82±0.52	3.00±0.15	3.18±0.15	3.20±0.16	2.98±0.12	2.88±0.52	3.04±0.44
Total thermophilic anaerobes	2.93±0.40	3.13±0.09	2.93±0.52	3.19±0.09	3.20±0.18	3.07±0.16	3.12±0.06	3.26±0.03	2.93±0.54	3.12±0.12	3.23±0.02	3.19±0.09
Yeast and Molds	2.95±0.34	3.10±0.28	3.35±0.20	3.14±0.20	3.20±0.21	3.20±0.18	3.13±0.28	3.22±0.17	3.38±0.06	3.22±0.14	3.13±0.34	3.18±0.12
Coliforms (log MPN/g)	3.15±0.22	3.30±0.15	3.29±0.06	3.36 + 0.15	3.35±0.12	3.17±0.15	3.28±0.15	3.38±0.00	3.29±0.05	3.17±0.16	3.20±0.37	3.37±0.18
Fecal Coliforms (log MPN/g)	3.43±0.44	3.37±0.08	3.32±0.02	3.62 + 0.52	3.45±0.06	3.43±0.44	3.37±0.08	3.46±0.03	3.37±0.01	3.44±0.44	3.50±0.30	3.68±0.52
Mesophilic sporeforming aerobes	3.54±0.55	4.01±0.48	4.07±0.46	3.69±0.12	4.13±0.44	3.76±0.15	4.07±0.48	4.31±0.12	4.20±0.14	3.80±0.15	3.63±0.43	3.73±0.12
Mesophilic sporeforming anaerobes	3.79±0.16	3.95±0.31	3.94±0.07	4.03±0.18	4.05±0.08	3.82±0.05	3.98±0.28	4.08±0.08	3.98±0.05	3.87±0.05	3.79±0.16	3.99±0.18
Thermophilic sporeforming aerobes	0.75±0,00	0.75±0.40	1.00±0.00	0.58±0.40	1.00±0,00	0.58±0.40	0.59±0.00	0.50±0.00	0.75±0.00	0.67±0.40	0.67±0.00	0.50±0.40
Thermophilic sporeforming anaerobes	0.58±0,00	0.92±0.00	0.92±0.00	0.50±0.00	0.92±0,00	0.58±0.00	0.92±0.00	0.92±0,00	0.95±0.00	0.75±0.00	0.58±0,00	0.50±0.00
Year 2002 Groups of Microorganisms	**January Mean±CI**	**February Mean±CI**	**March Mean±CI**	**April Mean±CI**	**May Mean±CI**	**June Mean±CI**	**July Mean±CI**	**August Mean±CI**	**September Mean±CI**	**October Mean±CI**	**November Mean±CI**	**December Mean±CI**
Total mesophilic aerobes	6.78±0.18	6.71±0.20	7.06±0.26	7.18±0.00	7.01±0.12	6.64±0.58	7.18±0.22	7.20±0.04	7.01±0.02	6.79±0,01	7.04±0.28	6.89±0.40
Total mesophilic anaerobes	5.03±0.24	5.89±0.78	5.45±0.72	6.21±0.14	6.18±0.14	5.20±0.20	6.07±0.58	6.15±0.74	6.08±0.80	5.42±0.68	5.55±0.78	5.65±0.40
Total thermophilic aerobes	2.92±0.52	3.08±0,00	3.00±0.40	2.85±0.54	3.10±0.34	2.74±0.38	3.01±0.54	2.92±0.58	2.96±0.32	2.96±0.37	3.20±0.10	3.09±0.06
Total thermophilic anaerobes	2.94±0.34	3.04±0.56	2.95±0.52	3.11±0.12	3.13±0.03	3.25±0.00	3.27±0.01	3.16±0.06	3.11±0.12	3.22±0.18	3.17±0.06	3.15±0.18
Yeast and Molds	3.19±0.18	3.56±0.19	3.13±0.38	3.31±0.08	3.28±0.22	3.26±0.15	3.22±0.10	3.27±0.15	3.38±0.08	3.20±0.21	3.28±0.00	3.46±0.15
Coliforms (log MPN/g)	3.34±0.15	3.37±0.03	3.27±0.00	3.29±0.02	3.31±0.02	3.40±0.15	3.41±0.18	3.37±0.18	3.25±0.20	3.37±0.12	3.43±0.05	3.29±0.18
Fecal Coliforms (log MPN/g)	3.44±0.00	3.28±0.08	3.30±0.35	3.44±0.19	3.71±0.16	3.62±0.52	3.68±0.52	3.68±0.51	3.44±0.18	3.45±0.06	3.69±0.38	3.50±0.23
Mesophilic sporeforming aerobes	4.15±0.28	4.10±0.52	4.09±0.38	3.86±0.03	3.56±0.26	3.71±0,04	3.74±0.12	3.73±0.12	3.93±0.02	4.15±0.44	3.82±0.14	3.84±0.03
Mesophilic sporeforming anaerobes	4.07±0.04	4.05±0.22	3.94±0.34	3.77±0,29	3.91±0.00	4.06±0.20	4.02±0.12	3.94±0.06	3.71±0.00	4.06±0.02	4.04±0.16	3.85±0.05
Thermophilic sporeforming aerobes	0.67±0.40	0.75±0.40	0.42±0.40	0.58±0.00	0.33±0.40	0.33±0.00	0.25±0.00	0.42±0.00	0.42±0.40	0.92±0.00	0.75±0.40	0.67±0.40
Thermophilic sporeforming anaerobes	0.67±0,00	0.58±0.00	0.67±0.00	0.58±0.00	0.58±0.00	0.83±0.00	0.58±0.40	0.50±0.00	0.92±0.00	0.92±0.00	0.50±0.00	0.58±0.00

CI = confidence interval (n = 12; p<0.05).
MPN = most probable number.

Table 6 - Microbial characteristics of the canned product. The counts are expressed in decimal logarithms (log CFU/G).

Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	6.24±0.58	6.83±0.40	6.87±0.56	6.96±0.16	6.80±0.42	6.61±0.40	7.00±0.00	6.77±0.30	6.74±0.02	6.95±0.38	7.01±0.02	6.67±0.40
Total mesophilic anaerobes	5.91±0.56	5.48±0.42	5.42±0.72	5.13±0.10	5.60±0.40	5.68±0.72	5.81±0.52	5.37±0.80	5.67±0.15	5.85±0.30	6.14±0.12	6.06±0.38
Total thermophilic aerobes	1.42±0.80	1.17±0.00	1.33±0.00	1.50±0.40	0.58±0.40	1.08±0.40	1.67±0.00	0.92±0.40	1.25±0.80	1.17±0.40	1.50±0.40	1.50±0.40
Total thermophilic anaerobes	1.58±0.00	1.08±0.40	1.58±0.00	1.00±0.80	1.08±0.40	1.25±0.40	1.33±0.00	0.92±0.80	1.25±0.80	1.42±0.00	1.33±0.40	1.50±0.00
Yeast and Molds	2.87±0.40	3.03±0.28	3.24±0.40	3.39±0.12	3.40±0.32	3.29±0.45	3.44±0.20	3.11±0.12	3.47±0.52	3.46±0.20	3.52±0.20	3.04±0.40
Coliforms (log MPN/g)	3.17±0.03	3.17±0.03	3.28±0.00	3.31±0.00	3.33±0.00	3.31±0.00	3.32±0.03	3.32±0.00	3.32±0.00	3.37±0.03	3.32±0.03	3.17±0.03
Fecal Coliforms (log MPN/g)	3.26±0.00	3.32±0.03	3.18±0.12	3.32±0.00	3.29±0.00	3.34±0.04	3.28±0.03	3.36±0.00	3.35±0.03	3.31±0.03	3.27±0.03	3.34±0.00
Mesophilic sporeforming aerobes	4.19±0.12	4.28±0.00	4.33±0.00	4.41±0.00	4.46±0.04	4.40±0.04	4.41±0.00	4.41±0.03	4.39±0.02	4.47±0.02	4.43±0.00	4.23±0.06
Mesophilic sporeforming anaerobes	4.20±0.12	4.19±0.04	4.25±0.04	4.28±0.02	4.28±0.02	4.24±0.40	4.29±0.08	4.22±0.10	4.27±0.00	4.30±0.02	4.30±0.08	4.22±0.06
Thermophilic sporeforming aerobes	0.83±0.00	0.67±0.40	0.67±0.00	0.67±0.00	0.67±0,40	0.75±0.00	0.58±0.00	0.75±0.40	0.50±0.40	0.92±0.00	0.42±0.00	0.17±0.00
Thermophilic sporeforming anaerobes	0.67±0.00	0.67±0.00	0.67±0.00	0.58±0.00	0.50±0.40	0.42±0.40	0.67±0.00	0.67±0,40	0.50±0.40	0.58±0.40	0.58±0,00	0.67±0.00
Year 2002 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	6.91±0.18	6.95±0.02	7.15±0.08	7.06±0.02	6.65±0.16	7.14±0.00	7.14±0.05	7.01±0.06	7.00±0.00	7.05±0,00	7.07±0.02	7.05±0.00
Total mesophilic anaerobes	5.50±0.76	5.90±0.46	5.88±0.32	5.99±0.35	6.10±0.38	5.46±0.30	5.53±0.76	5.90±0.62	6.02±0.32	5.70±0.10	5.96±0.52	5.67±0.70
Total thermophilic aerobes	1.42±0.80	1.83±0,00	1.17±0.40	1.58+ 0.40	1.58±0.40	1.50±0.40	1.33±0.80	1.58±0.00	1.83±0.40	1.50±0.00	1.67±0.00	1.67±0.00
Total thermophilic anaerobes	1.42±0.80	1.58±0.40	1.42±0.40	1.42±0.00	1.42±0.40	1.42±0.80	1.33±0.80	1.67±0.40	1.67±0.40	1.33±0.80	1.50±0.40	1.42±0.40
Yeast and Molds	3.53±0.32	3.49±0.08	3.49±0.20	3.50±0.04	3.09±0.18	3.35±0.00	3.57±0.32	3.48±0.04	3.52±0.08	3.34±0.03	3.56±0.10	3.24±0.52
Coliforms (log MPN/g)	3.33±0.00	3.31±0.03	3.36±0.00	3.33±0.00	3.17±0.03	3.35±0.00	3.36±0.00	3.35±0.00	3.36±0.00	3.32±0.00	3.34±0.03	3.33±0.03
Fecal Coliforms (log MPN/g)	3.36±0.03	3.29±0.03	3.31±0.00	3.29±0.03	3.38±0.00	3.32±0.00	3.37±0.03	3.32±0.03	3.30±0.00	3.31±0.00	3.32±0.00	3.31±0.03
Mesophilic sporeforming aerobes	4.43±0.04	4.41±0.00	4.45±0.02	4.44 + 0.00	4.25±0.06	4.45±0,06	4.40±0.04	4.45±0.40	4.43±0.02	4.43±0.06	4.42±0.02	4.42±0.00
Mesophilic sporeforming anaerobes	4.29±0.00	4.28±0.08	4.32±0.00	4.31±0,08	4.22±0.06	4.29±0.00	4.30±0.06	4.33±0.14	4.29±0.08	4.29±0.02	4.34±0.14	4.33±0.14
Thermophilic sporeforming aerobes	0.83±0.40	0.50±0.40	0.75±0.00	0.58±0.40	0.50±0.40	0.50±0.00	0.58±0.00	0.58±0.40	0.67±0.00	0.58±0.00	0.67±0.40	0.50±0.40
Thermophilic sporeforming anaerobes	0.67±0,40	0.67±0.00	0.42±0.40	0.67±0.00	0.67± 0.00	0.50±0.00	0,67±0,40	0.67±0.00	0.67±0.00	0.67±0.00	0,67±0.00	0.67±0.00

CI = confidence interval (n = 12; p<0.05).
MPN = most probable number.

1986; KELLER *et al.*, 2002). Microorganisms from the raw materials accumulate on the equipment and work surfaces, and are therefore distributed to all products passing through the system (MOORE and GRIFFITH, 2002).

Retorting canned meat analog involves stabilizing the heating and cooling temperatures of the product in pressurized cookers or autoclaves. The shelf-life of this canned product is established during hygienic processing and the final thermal treatment. In spite of the high mesophilic counts (including spore formers) in the canned product, the autoclaved cans, which were incubated for 10 days at 35°C (ANVISA, 2001), proved that the autoclaving was sufficient and the product attained commercial sterility, with no detection of any of the microorganisms analyzed. In Brazil, the directive for processed food in hermetic packing, which must be stable at room temperature, has not established a microbiological standard for the vegetable protein product (meat substitute) or at any stage of the process, including the canned products before the autoclaving operation.

The present study describes the importance of microbial control of the raw vegetable proteins and at all processing stages. Due to the high levels of microorganisms in the soy protein raw material, exposure of this protein to the optimal growth conditions during processing caused a considerable increase in the bioburden of the canned product, previous to moist heat treatment.

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