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Testing a Small Scale Aseptic System for Milk in Plastic Bottles

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Abstract The objective of this study was to develop and assess the performance of an aseptic system for liquid milk contained in plastic bottles, from a small-scale production standpoint. Commercial sterility tests conducted on the bottled milk were utilized in our assessments of the system, via the identification and monitoring of the principal points of the process. Four 150 L batches of milk with pH values of approximately 6.7 were heat-processed at between 137 and 143°C for 10 sec in a plate heat exchanger, and then aseptically transferred to 500 mL high-density polyethylene (HDPE) bottles, in an ISO class 7 clean room. The aseptic condition of the bottles was achieved via 10 sec of rinsing with a mixture containing 0.5% peracetic acid and 0.8% hydrogen peroxide at 30°C, followed by another rinse with sterile water. Of the 4 batches processed, 2 were determined to exhibit commercial sterility, on the basis of the physical-chemical and microbiological criteria adopted. It was concluded that some adjustment of the processing line was required in order to achieve full commercial sterility for all processes. The aseptic system developed and assessed in this study was demonstrated to have great potential for the processing and transferring of milk into plastic bottles, from a small-scale production standpoint.

Keywords: UHT milk, aseptic system, plastic bottle

Introduction

Traditional food sterilization methods have been determined to be unsatisfactory for milk, as they tend to impart a 'cooked' taste, and also cause some deterioration in the nutritional value of the milk (1). Thus, the quality of milk processed via ultra-high temperature (UHT) tends to be superior to that of milk that is sterilized in its packaging, as the UHT milk is exposed to high temperatures for only a few seconds. UHT treatment involves the heating of the milk to temperatures between 135 to 150°C for 1 to 10 sec followed by rapid cooling, allowing for the achievement of commercial sterility with a minimum of chemical alterations to the product (2).

The UHT system has become a widely utilized and easily commercialized product. It allows, within the industrial sector, for the transport and sale of the product at distant destinations without any need for refrigeration, which had previously proven impossible due to climatic factors. This is clearly beneficial in cases in which a product is commercialized in a large country (3).

Over the years, milk has been subjected to a great variety of different processes targeted toward the augmentation of its shelf life, and many kinds of packaging systems have also been attempted. The aseptic filling of plastic bottles has become an important approach, due to the great advantages it presents as compared to other systems, although the hygiene procedures inherent to this approach are far more exacting than for other filling processes.

In aseptic processes, the objective of the heat treatment is the inactivation of microorganisms, particularly mesophilic spores (4). Commercially sterile milk is a product with many attractive aspects, most notably longer shelf life without need for refrigeration, a result of the heat treatment applied during processing.

Aseptic filling demands a great deal more rigid hygiene control than is required for other packaging processes, primarily due to microorganismic spores that are present in the environment, which may recontaminate the product. Thus, adequate control of the processing conditions and package asepsis are imperative for desired industrial efficiency.

In view of the considerable current demand for an aseptic procedure for the transfer of milk into plastic bottles in small-scale production schemes, this study was conducted in order to ascertain the efficiency of a newly-developed aseptic system for small-scale food industrial applications.

Material and Methods

Process description ***Heat treatment***: Four 150 L batches of milk were processed using a plate heat exchanger in accordance with the binomials displayed in Table 1. The milk was cooled to 25°C immediately following heat treatment, then pumped into an aseptic container in a clean room and transferred to clean 500 mL high density polyethylene (HDPE) bottles with polyethylene (PP) closures.

Package asepsis: The plastic bottles were sterilized by spraying a stabilized mixture of hydrogen peroxide and peracetic acid into the bottles, followed by a rinse with sterile water. The seals (aluminum/low density polyethylene, Al/LDPE) attached to the closures were immersed in the same solution for 2 min. The concentration-temperature-time trinomial of the peracetic acid (PAA) used for bottle asepsis was 0.5% PAA/30°C/10 sec (5).

Aseptic filling: The aseptic filling of the product was conducted in an ISO class 7 clean room with simultaneous

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Table 1. Binomials used on the heat treatment of the milk batches using a plate heat exchanger and total particles count of the air in the clean room during the filling operation of UHT milk

Batches	Binomials	Count of particles[1] (particles/m^3 of air)	
		≥0.5 μm	≥5.0 μm
B_1	137°C/10 sec	29333	<1000
B_2	141°C/10 sec	48000	<1167
B_3	143°C/10 sec	27333	<1000
B_4	138°C/10 sec	30833	<1000

[1]Mean values from the beginning, middle and from the end of the filling operation.

monitoring of the total particles and microorganisms suspended in the air. After filling, the bottles were heat-sealed by induction, closed with screw-on polyethylene caps, and labeled on the basis of processing batch and manufacturing date.

Process flow sheet: Figure 1 shows the flow sheet employed in the processing of the 4 batches, which was differentiated only in terms of the processing temperature.

Checking filling room and bottles: The efficiency of the packaging system was assessed via the monitoring of the operating conditions of the filling room and by counting the numbers of microorganisms within the bottles. The air flow rate in the clean room and its positive pressure were also measured. The asepsis efficiency of the bottles was confirmed by sampling the bottles during milk processing for microbial counts (6).

Product evaluation Three hundred 500 mL bottles were prepared for each batch, for a total of 150 L of bottled milk. Three bottles were separated at the end of each processing for pH determination and evaluations of the microbiological stability of the produced batches of milk.

Sample pre-incubation: At the end of processing, 60 bottles from each batch were separated-20 from the beginning of processing, 20 from the middle, and 20 from the end (7, 8). The bottles were then incubated at 35°C for 10 days, and the remaining bottles were stored at room temperature (25-30°C).

The pre-incubation technique was developed in an

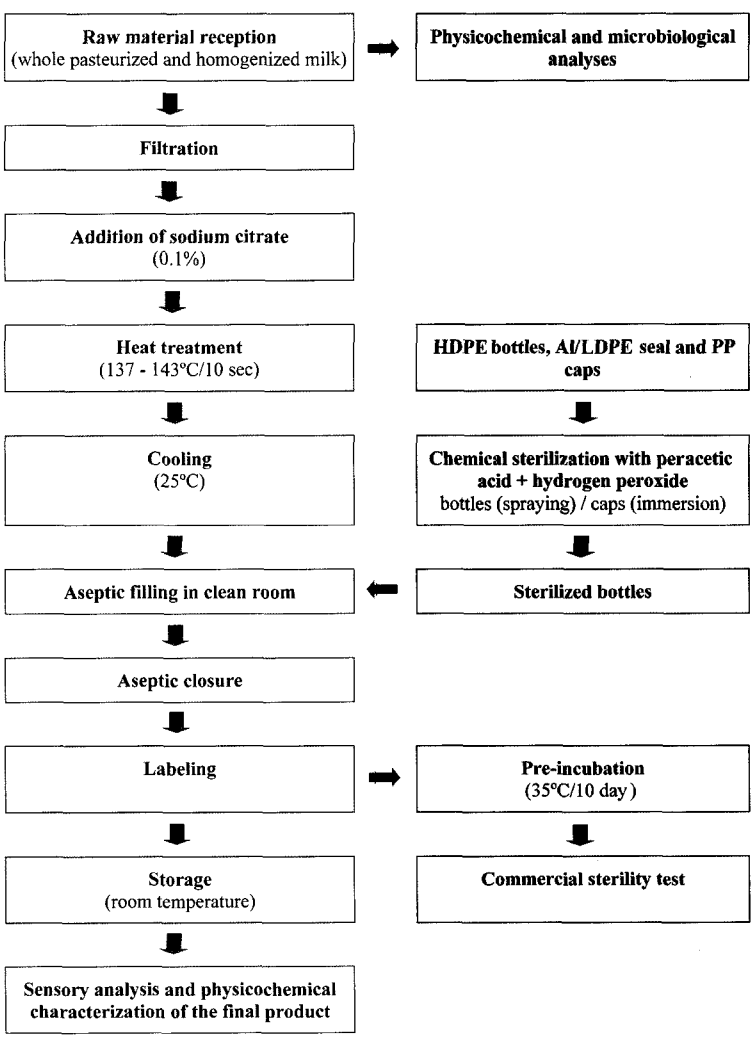

Fig. 1. Flow sheet of the milk aseptic processes.

attempt to increase the number of viable bacteria or spores, if present. The normally recommended conditions vary from 30 to 35°C for 7 to 10 days. Under these conditions, if the product acidifies or coagulates, the presence of viable microorganisms is established (9).

Visual inspection of the batches: A visual inspection of the produced batches was conducted during and at the end of the pre-incubation period, with checks made for visual alterations such as package swelling. After the bottles were opened, changes in color, odor, viscosity, and texture were investigated immediately prior to the determination of pH.

pH determination: After the incubation period, the pH values of the 60 units of each batch were determined. A variation of less than 0.2 pH units in relation to the determination made immediately after processing was tolerated (10). Of the 60 units of each incubated batch, 3 were submitted to microbiological analyses, in order to detect eventual contaminants.

Microbial analysis: The procedure employed to determine contaminants was conducted according to (6). The package could not be opened in the conventional way if the integrity of the opening system was to be guaranteed, and thus the area to be perforated in order to remove the sample was disinfected with iodated alcohol for 20 min prior to sampling.

After the contents were homogenized, 2 mL aliquots were removed using a graduated syringe with a sterile needle to perforate the bottle, and were transferred to screw-top tubes containing bromocresol purple dextrose broth for the aerobic test, and cooked meat medium for the anaerobic test, the latter being sealed with vaspar (vaseline/paraffin).

After incubation, all inoculated tubes were observed at 35 and 55°C for 2 to 5 days, checking for microbiological development as indicated by the turbidity of the medium, the formation of a pellicle on the surface, and/or gas production. The bromocresol purple dextrose broth tubes were also evaluated for acid production, which would have been evidenced by a change in the color of the medium to yellow. The absence of change in all tubes indicated that the product could be considered commercially sterile. However, the turbidity of the milk made the observation of microbial growth in the tubes difficult, and thus confirmation by transference to streak plates was required. The plates, which contained manganese nutrient agar and calf liver agar, were incubated at the same temperature as was the original tube. After an incubation period, any microbial development on the streak plates verified the occurrence of development within the original tube.

Results and Discussion

Packaging system evaluation ***Pressure of filling room***: It was observed that the filling room was operated under the correct conditions. The pressure differential between the inside and outside rooms was 2.8 mmH_2O, a value twice that recommended by the US Food and Drug Administration (FDA IES-PP-CC 00-2).

Particle count: The results of the filling room particle count are shown in Table 1. Such values were in accordance with ISO 14644-1 for Class 7, the condition recommended for the operation of aseptic systems (4). The use of a clean filling room rendered the processed milk less likely to re-acquire environmental microorganisms, thus making it more likely that commercial sterility could be achieved.

Air microbial count: The viable microbial count in the air was monitored during the processing operation. Table 2 shows the number of microorganisms in the air at the beginning, middle, and end of the 4 batches of UHT milk production. A low microbial load was observed in the filling room air, the values of which were below that recommended for UHT milk processing (11, 12). Pharmaceutical industries also operate in ISO class 7, where the number of microorganisms in the air is normally less than 20 CFU/m^3. Accordingly, Table 2 also shows the small amount of mould spores measured in the filling room. Mean values of 2.8×10^2 CFU/m^3 have been determined for milk plants (13). In accordance with (14), the mean values in clean rooms for aseptic packaging must be less than 1.8×10^3 CFU/m^3. This value also depends on the diameter of the bottle finish, and a maximum of 38 CFU/m^3 is recommended for a diameter of approximately 4 cm, and the filling time is up to 10 sec (11).

Bottle sterilization: The evaluation criteria utilized to check the bottles was conducted by counting the total microbial content of the sterilized bottles. The mean initial microbial count was determined to be 8 CFU/bottle, and the value was zero after chemical sterilization. This level of system efficiency confirms the results shown in (5). Also, the microbial load was not particularly high, considering that the recommended value is less than 20 CFU/bottle for most aseptic systems (15). The residual H_2O_2 observed in the bottles after rinsing with sterilized water was less than 0.5 mg/L, a level which is in compliance with FDA regulations (16).

Milk stability evaluation ***Visual inspection of batches after pre-incubation***: The 60 sampled bottles of each batch were visually inspected after 10 days of incubation

Table 2. Plate microbial count and mould count of the air in the clean room during aseptic filling of UHT milk

Batches	Microbial count (CFU/m^3 of air)				Mould count (CFU/m^3 of air)			
	Begin	Middle	End	Mean	Begin	Middle	End	Mean
B_1	<2.0	<2.0	2.0	<2.0	<2.0	<2.0	<2.0	<2.0
B_2	4.0	4.0	<2.0	<3.3	2.0	4.0	2.0	2.7
B_3	2.0	4.0	<2.0	<2.7	2.0	<2.0	2.0	<2.0
B_4	<2.0	<2.0	4.0	<2.7	4.0	2.0	4.0	3.3

at 35°C, and the results were as follows:
Batch 1 - changes in color, odor, or swelling not observed
Batch 2 - no apparent alterations
Batch 3 - 2 units evidenced acidic or fermented odor, with clear alterations in product viscosity and texture.
Batch 4 - 1 unit evidenced acidic or fermented odor, with clear alterations in product viscosity and texture.

pH determination: After pre-incubation, the pH value of each of the 60 units of each batch was determined. In accordance with the work of Cerf (10) the concept of the stability of UHT milk presupposes pH fluctuations not greater than 0.2. Based on that protocol, the following considerations were made:
Non-sterility rate
Batch 1 - of the 60 units evaluated, no significant pH change ($\Delta pH>0.2$) in relation to the value immediately after processing.
Batch 2 - no unit with any significant pH change.
Batch 3 - 2 units with a significant pH change.
Batch 4 - 1 unit with a significant pH change.
The percentage of non-sterile bottles in relation to the 60 bottles incubated, were as follows: 0, 0, 3.33, and 1.67%, for batches 1, 2, 3, and 4, respectively.

Microbiological analysis for the detection of contaminants After the pre-incubation of the samples at 35 °C/10 day, 3 units from each batch were subjected to a procedure for the detection of contaminants, indicating the presence or absence of microorganisms under specific incubation conditions (6). Table 3 shows the final diagnoses of the milk samples.

The results have shown confirmed the presence of deteriorating microorganisms in the milk, which had been indicated previously by the pH determinations, due to the appearance of fluctuations in excess of 0.2. That is, batche 3 and 4, which harbored samples with reduced pH, were re-confirmed to have been contaminated in the microbiological tests, by virtue of the detection of mesophiles.

In an aseptic system, contamination may occur during product sterilization, post-sterilization, during filling, and following packaging. The occasional presence of spore-forming microorganism resistant to UHT treatment must also be considered (17, 18).

The aseptic system assessed in this study have evidenced potential for the processing and filling of milk into plastic bottles on a small scale. However, some adjustments to the filling equipment also appear necessary. These improvements will be required in order to prevent possible product recontamination, and also to achieve a small bottle headspace, which will augment the shelf life of the milk.

Table 3. Final diagnosis for the milk samples aseptically processed and filled into PEAD bottles

Batches	Milk sample[1)]	Classification of microorganism[2)]	Final sample[3)]
B_1	S_1	-	cs
	S_2	-	cs
	S_3	Aerobic thermophile	cs
B_2	S_1	-	cs
	S_2	-	cs
	S_3	Aerobic thermophile	cs
B_3	S_1	-	cs
	S_2	Aerobic mesophile	ncs
	S_3	Aerobic thermophile	cs
B_4	S_1	Facultative anaerobic Mesophile/thermophile	ncs
	S_2	-	cs
	S_3	-	cs

[1)] $S_1/S_2/S_3$ - samples taken at the beginning, middle, and end of processing, respectively.
[2)] – no microbial development.
[3)] cs - commercially sterile; ncs - not commercially sterile.

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