EFFECT OF PASTEURIZATION TEMPERATURE AND ASEPTIC FILLING ON THE SHELF-LIFE OF MILK*

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ABSTRACT: Aseptic filling process can contribute to extend the shelf-life of pasteurized milk. However, this technology is not still commercially practiced in Brazil for this product. The aim of this study was to evaluate the effect of the pasteurization temperature and aseptic filling on the stability of milk packaged in high density polyethylene bottle (HDPE). Three batches of one hundred bottles each were processed at 72, 83, and 94ºC/15s, followed by aseptic packaging and storage at 9ºC, simulating a deficient cold chain scenario as found in the Brazilian market. Microbiological stability evaluation was based on mesophilic and psychrotrophic counts. Sensory shelf-life was estimated according to the ratings assigned to the attributes appearance, aroma, flavor and overall appreciation of milk samples. The pasteurization temperature, in the range investigated, did not affect the shelf-life of milk, which lasted up to 11 days for the three processed batches. The aseptic filling, associated to the employed thermal treatments, did not extend the shelf-life of milk stored under the abuse condition of 9ºC, considering that the commercial product shelf-life in Brazil is of about 10 days.

KEYWORDS: Food stability; pasteurized milk; aseptic filling; sensory analysis.

INTRODUCTION

There is no universal definition of milk product shelf-life nor is one method generally recognized for determining it. Shelf-life describes the duration, in days, following processing that milk is of acceptable quality. Since consumer acceptance is the ultimate factor determining shelf-life, a consumer or trained sensory panel may be used to determine a food shelf-life. Pasteurized milk must be stored under refrigeration and has a relatively short shelf-life. In Brazil, according to some processors, the shelf-life of pasteurized milk ranges from 3 to 10 days due to the low quality of the raw material and the precarious cold storage chain which exposes the product to improper conditions of temperature during distribution and commercialization.

Microbial spoilage of pasteurized milk is of concern since milk supports abundant growth of microorganisms, leading to development of off-flavors, coagulation and ropiness. The time required for flavor changes to occur depends on the initial numbers and types of microorganisms present, the pasteurization conditions and the storage temperature. Furthermore, post pasteurization recontamination is also a factor of concern.9

Bacterial spoilage is the most important limiting factor in extending the shelf-life of conventional – high temperature short time (HTST) – pasteurized milk beyond 14 days. Microbial growth and metabolism shorten the shelf-life of milk by producing undesirable changes in aroma and taste attributes that influence consumer acceptability of the food product.7

Sepulveda et al.21 found that under ideal conditions of processing and storage, pasteurized milk can reach a shelf-life of three weeks depending on the microbiological quality of the raw milk. According to Rysstad & Kolstad,19 the shelf-life of pasteurized milk can be extended by introducing better hygienic transfer from processing to filling machines and the filling process.

As far as packaging material concerns, high density polyethylene (HDPE) is a versatile plastic with many uses, especially for food packaging. This plastic, which carries low risk of leaching and which is readily recyclable, is commonly used for packaging pasteurized milk and an array of high-added-value dairy beverages. The main advantages of aseptically filled plastic bottles as compared to laminated cartons include the possibility of producing the packages on the same premises where the milk is processed; access to a wider choice of suppliers and thus more competitive options; development of custom-made bottles of multiple shapes and sizes, creating an opportunity to build a differentiated brand image; transparent container walls through which the product in the container can be viewed directly; and the practical plastic containers are easy to handle, open and close. In the particular case of monolayer HDPE, there are additional advantages of low-cost and ready recyclability. On the other hand, the drawbacks of monolayer HDPE
are its high oxygen permeability and low light barrier properties, which may cause nutritional losses and bring about undesirable changes in the sensory characteristics of the product during storage.6,14 However, the use of HDPE may be profitable when a short or intermediate shelf-life is intended.

Considering that aseptic filling process is not practiced for pasteurized milk in Brazilian market, the purpose of this study was to evaluate the effect of this technology on the shelf-life of this product packed in HDPE bottles, submitted to three pasteurization temperatures. In addition, in order to verify the efficiency of aseptic filling on keeping quality of pasteurized milk submitted to inadequate storage conditions, a temperature storage of 9°C was employed, simulating a deficient cold chain scenario as found in the Brazilian market.

MATERIAL AND METHODS

The milk processing and analyses presented in this work, related to pasteurization (72, 83 and 94°C) and storage temperatures (9°C), were obtained from assays based on a statistical factorial design, with three replicates for the central point (83°C), as described by Petrus et al.15

Milk Processing

Raw milk, machine-milked and chilled about 3h before processing, was transported in an isothermal vehicle from the dairy barn located 120m away from the pilot plant at the Faculty of Animal Science and Food Engineering, University of São Paulo/SP - Brazil. Three individual batches, pasteurized at 72, 83, and 94°C/15s, resulted in one hundred units each of 500 mL pigmented TiO2 PEAD bottles. Of the 100 units, 50 units were stored at 9°C and 50 units were stored at 0°C used as control samples in sensory tests. Figure 1 shows the flowchart of milk processing and packaging.

Physicochemical Analyses

Physicochemical analyses were carried out in compliance with Adolfo Lutz Institute (IAL) official methods for fluid and pasteurized milk and according to the official methods for milk and milk products established by the Brazilian Regulation, Instrução Normativa n. 68, 12/12/2006/MAPA - Ministério da Agricultura, Pecuária e Abastecimento. 1, 15 Analyses (triplicates) were carried out as follows: density (specific gravity), fat matter (g/100g), dry matter, no fat dry matter (g/100g), pH, acidity (g lactic acid/100mL), protein as total nitrogen, 72% alizarol stability (v/v), peroxidase, and alkaline phosphatase.

Criteria for Estimating Shelf-life of Pasteurized Milk

The first microbiological and sensory analysis (time 0 of storage) was carried out a couple of hours after milk processing and aseptic bottling.

Microbiological tests

The total mesophilic bacterial population present in thermal treated samples was considered to be the main index defining the shelf-life of milk in this study since standard plate count is one of the most commonly used tests to evaluate the quality of freshly pasteurized milk.17

Three pasteurized milk samples (three per treatment), aseptically filled in HDPE bottles, were analyzed for the presence of aerobic mesophilic and psychrotrophic bacteria in order to estimate the microbiological stability of the product. Mesophilic counts of 7log CFU/mL (107 CFU/mL) and psychrotrophic of 6 log CFU/mL (106 CFU/mL) were the maximum accepted levels for determining the quality of pasteurized milk, according to Moyssiadi et al., 13 Cromie, 4 and Craven & Macauley. 3 These bacterial levels are associated with unacceptable flavor in pasteurized milk.24

Sensory tests

The sensory stability of the milk batches was estimated based on sensory results of a panel consisting of 30 untrained and habitual consumers of pasteurized milk. The panelists were asked to evaluate the sensory attributes of appearance, aroma, flavor, and overall appreciation by assigning a liking score on a 7-point hedonic scale (1=dislike very much; 4=neither like nor dislike; 7=like very much). 25 The tests were accomplished in individual booths lightened with a fluorescent white lamp, and the samples were monadically presented in 50mL plastic cups labeled with a 3-digit code, at a temperature of about 17°C. One milk sample, obtained from the same production batch, stored at 0°C, was taken to serve as control. Mineral water was provided to cleanse the palate. The average scores > 4 and percentage of approval > 50% were used as threshold values for all the attributes assessed.

In addition, difference-from-control test was carried out in order to determine whether a difference existed between the milk sample (stored at 9°C) and the control (stored at 0°C) during the storage time, and to estimate the size of any such difference. A panel of 30 subjects were asked to rate the size of the difference between the sample and the control by assigning a score on a 5-point scale (0=no difference between sample and control; 2=moderate difference; 4=very large difference).11 Milk samples were served to the panel in the same conditions that those described for hedonic scale test.

Statistical Analysis

The results were submitted to analysis of variance, Tukey’s test with significance set a priori at 5%. Difference-from-control sensory test results were submitted to Dunnett’s test using the statistical software program SAS 9.1.
RESULTS AND DISCUSSION

Physicochemical Analysis

The results of the physicochemical analyses performed on samples of pasteurized milk are depicted in Table 1.

All raw milk samples showed physical and chemical good quality, confirmed by means of absence of coagulation in alcohol stability assay, pH values (from 6.58 to 6.76) and by an acidity level (from 0.142 to 0.167% w/v of lactic acid).

The same procedures were applied soon after each processing batch. All pasteurized samples showed stability in 72% alcohol. Alkaline phosphatase results were negative for all pasteurized samples, demonstrating the efficiency of pasteurization conditions tested. Peroxidase tests results were negative for the batch pasteurized at 94°C/15s only. Levels of no fat dry matter and protein were slightly inferior to those presented in the Brazilian official regulations (Table 1).

The results obtained and information available for Holstein cows treated in Brazil, suggest that the chemical composition of milk is related to breed, age, stage of lactation, and animal handling, such as feeding quality.

Shelf-life Assessment

Microbiological stability

It is quite known that contamination of raw milk can occur during the several steps of processing, including hygiene procedures (water quality) and inadequate refrigeration. Several Brazilian researches point out deficiencies in the milk chain, assigning the refrigeration and milk production systems steps. Pinto et al. have found counts from $2.5 \times 10^3$ (3.40 log CFU/mL) to $3.0 \times 10^6$ (6.48 log CFU/mL) and from $2.0 \times 10^2$ (2.30 log CFU/mL)
to 1.0x10^7 CFU/mL (7.00log CFU/mL), for mesophiles and psychrotrophic bacteria, respectively, in refrigerated raw milk.

Mesophilic and psychrotrophic means counts in raw milk observed in this research were 4.58 and 3.83log CFU/mL, respectively. Psychrotrophic counts were higher than 10% of mesophilic counts, maximum level established by Brazilian regulations, indicating that the milking hygienic procedures must be improved.

Mesophilic counts of homogenized whole pasteurized milk aseptically filled in HDPE bottles stored at 9°C are shown in Table 2.

Moysiaidi et al.\textsuperscript{13} and Karatapanis et al.\textsuperscript{20} evaluated the stability of aseptically filled milk pasteurized at 75°C/15s and stored in monolayer HDPE bottles pigmented with TiO\textsubscript{2}, the same packaging material used in this research. At time 0, mesophilic counts were above 4log CFU/mL, and after 7 d of storage at 4°C, the counts increased to 6log CFU/mL. Zygoura et al.\textsuperscript{27} reported that aseptically filled pasteurized milk (75°C /15s) in HDPE monolayer bottles pigmented with TiO\textsubscript{2} had mesophilic counts from 4log CFU/mL (at time 0) to 7log CFU/mL after 7 d of storage at 4°C.

Mesophilic bacteria in the present study ranged from 2.28log CFU/mL at time 0 to 5.76log CFU/mL after 7 d of storage at 9°C. These results indicated the good processing quality, which resulted in important reduction of mesophilic counts and longer microbiological stability of the product (Table 2).

According to results showed in Table 2, in compliance with upper limit considered for mesophilic count, the stability of milk was estimated in 11 d for all pasteurization temperatures, when stored at 9°C.

Table 3 (footnotes) shows that psychrotrophic counts in raw milk reached 3.83log CFU/mL, a similar level to that obtained by Sepulveda et al.\textsuperscript{22} which was 3.25log CFU/mL. According to Stepaniak,\textsuperscript{24} the initial count of psychrotrophics in pasteurized milk can vary from 0.001 to more than 10^3 CFU/mL (3log CFU/mL). The higher counts of psychrotrophic bacteria associated to higher storage temperatures will cause a shorter shelf-life of the product.

Moysiaidi et al.\textsuperscript{13} and Karatapanis et al.\textsuperscript{10} evaluated the stability of pasteurized milk in HDPE bottles and showed that the psychrotrophic counts were higher than 3log CFU/mL at time 0. After 7 d of storage at 4°C, the counts had exceeded 5log CFU/mL.

Low psychrotrophic counts in milk are of paramount importance for its quality because the metabolic activity of these microorganisms makes biochemical alterations in the constituents of milk. Psychrotrophic bacteria in milk stored under refrigeration develop metabolic activities that culminate with alterations in the flavor, odor and the

### Table 1 – Physicochemical characterization of whole pasteurized and homogenized milk.

<table>
<thead>
<tr>
<th>Pasteurization temperature (°C)</th>
<th>Specific gravity 15°C (g/ml)</th>
<th>Fat matter\textsuperscript{(1)} (%w/w)</th>
<th>Dry matter (%w/v)</th>
<th>No fat dry matter\textsuperscript{(2)} (% w/v)</th>
<th>pH</th>
<th>Acidity\textsuperscript{(3)} (% w/v)</th>
<th>Protein (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>1.030±0.001</td>
<td>3.10±0.01</td>
<td>11.4±0.1</td>
<td>8.3±0.1</td>
<td>6.63±0.01</td>
<td>0.168±0.001</td>
<td>2.60±0.01</td>
</tr>
<tr>
<td>83</td>
<td>1.028±0.001</td>
<td>3.09±0.02</td>
<td>11.2±0.3</td>
<td>8.1±0.3</td>
<td>6.64±0.01</td>
<td>0.149±0.001</td>
<td>2.43±0.02</td>
</tr>
<tr>
<td>94</td>
<td>1.028±0.001</td>
<td>2.95±0.04</td>
<td>10.79±0.05</td>
<td>7.847±0.006</td>
<td>6.60±0.01</td>
<td>0.166±0.002</td>
<td>2.55±0.08</td>
</tr>
</tbody>
</table>

Identity and quality patterns for pasteurized milk according to Instrução Normativa 62 (“Regulamento Técnico” 2011): \textsuperscript{1} (1) minimum of 3.0, (2) at least 8.4g/100mL, (3) 0.14 a 0.18g lactic acid/100mL.

Pasteurization holding time: 15s.

Mean values of three replicates ± standard deviation.

Means followed by the same letter in the same column are not significantly different (P > 0.05).

### Table 2 – Mesophilic counts\textsuperscript{a} (log CFU/mL) of homogenized whole pasteurized milk aseptically filled in HDPE bottles stored at 9°C.

<table>
<thead>
<tr>
<th>Thermal treatment (°C)\textsuperscript{b}</th>
<th>Storage time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>83</td>
<td>7</td>
</tr>
<tr>
<td>94</td>
<td>13</td>
</tr>
</tbody>
</table>

\textsuperscript{a}mean values of three replicates ± standard deviation.

\textsuperscript{b}holding time: 15s.

means followed by the same lower case letter within rows (comparison between storage times) or by the same upper case letter within columns (comparison between pasteurization temperatures) are not statistically different (P > 0.05).

upper limit considered for keeping quality of milk: 7 log CFU/mL.

mean counts in raw milk: 4.58log CFU/mL.

d: days.
The quality of milk is reduced by 50%. The authors also mention that increase of storage temperature, the shelf-life of pasteurized milk is reduced by 50%. The authors emphasize that for every 2°C increase of storage temperature, the shelf-life of pasteurized milk is reduced by 50%. The authors also mention that aseptic transfer and packaging, can improve the shelf-life, provided that the temperature of distribution is below 6°C. At higher temperatures shelf-life is reduced due to the impact on the surviving flora.

Table 3 – Psychrotrophic countsa (log CFU/mL) of homogenized whole pasteurized milk aseptically filled in HDPE bottles stored at 9°C.

<table>
<thead>
<tr>
<th>Thermal treatment (°C)b</th>
<th>Storage time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>72</td>
<td>0.01 Ab±0.02</td>
</tr>
<tr>
<td>83</td>
<td>0.49 Ab±0.20</td>
</tr>
<tr>
<td>94</td>
<td>0.17 Ac±0.12</td>
</tr>
</tbody>
</table>

a mean values of three replicates ± standard deviation.

Sensory stability

The average acceptability ratings obtained using the 7-point hedonic scale are shown in Tables 4, 6, and 8, along with the percentage of consumers that approved the sample by assigning a rating ≥ 4.0. Tables 5, 7, and 9 present the results obtained using the difference-from-control test. When it was observed coagulation and/or an unpleasant odor in the samples immediately before they were served to the panelists, the sensory tests were stopped.

Table 4 shows that milk pasteurized at 72°C obtained high average ratings on a 7-point hedonic scale and percentage of approval > 60%. Therefore, milk lasted 11 days according to the sensory criteria established in this research.

According to average ratings (> 4.5 for all sensory attributes) and percentage of approval (≥ 60%), shown in Table 6, milk samples processed at 83°C/15s and stored at 9°C lasted 11 days. According to average ratings (> 4.5 for all sensory attributes) and percentage of approval (≥ 60%), shown in Table 6, milk samples processed at 83°C/15s and stored at 9°C lasted 11 days.

Table 8 shows that milk samples obtained similar acceptability to that depicted in Tables 4 and 6. According to acceptance tests the pasteurization temperature did not affect the sensory stability of milk stored at 9°C.

As quoted by Griffiths & Phillips, 8 Moyssiadis et al., 13 Cromie, 4 and Craven & Macauley, 3 levels of mesophilic and psychrotrophic counts close to 7 and 6log CFU/mL, respectively, compromise the sensory quality of pasteurized milk, confirming results obtained in the present study.
This study shows that the shelf-life of aseptically filled pasteurized milk was 11 days, for all conditions evaluated. In spite of that, an interesting behavior can be noticed. According to data presented in Table 3, one can notice lower counts for psychrotrophic bacteria, along the storage time, on samples treated at 83°C, compared to pasteurization carried out at 72°C. It would be expected a significant perception level of panelists in the difference-from-control-test on samples treated at a lower temperature, since the difference in counts were of one order of magnitude. Treatments carried out at 83 and 94°C showed significance at 11 days storage for the difference-from-control test applied, which is in accordance to microbiological results obtained for the storage period, especially considering psychrotrophic counts.

Shelf-life of Pasteurized Milk

The results obtained in this research showed that the pasteurization temperature, in the levels studied, did not affect the shelf-life of the product. Vatne & Castberg reported that if the processing temperature is increased to the range of 80 to 90°C, it will stimulate the growth of spores,
20 the shelf-life of pasteurized, homogenized milk was
results observed in this study did not con
and thus decrease the shelf-life of milk. However, the
decrease the inhibiting effect of antimicrobial compounds,
produce growth factors more readily available to the cells,
decrease the inhibiting effect of antimicrobial compounds,
increase the severity of pasteurization
treatments, did not extend the shelf-life of milk stored under
the abuse condition of 9 °C, considering that the commercial
life of pasteurized milk stored at 7°C varied from 7 to 8 d.
Simon & Hansen 23 stated that pasteurized milk (72
Stepaniak24 mentioned that the shelf-life of
Storage
Control1 mean Sample2 mean
7 1.17a 1.37a
11 0.97b 1.50a
Means followed by the same lower case letter within rows (comparison between sample and control) or by the same upper case lett er within columns (comparison between storage times) are not statistically different (P > 0.05). 1stored at 0ºC. 2stored at 9ºC. Scale: (0) no difference between sample and control, (1) slight difference, (2) moderate difference, (3) large difference, and (4) very large difference.

CONCLUSION
This survey showed that pasteurization temperature,
ranged from 72 to 94°C/15s had no effect on the microbiological and sensory shelf-life of aseptically packed milk into plastic bottle, which lasted 11 d under storage at 9°C. The aseptic filling, associated to the employed thermal treatments, did not extend the shelf-life of milk stored under the abuse condition of 9 °C, considering that the commercial product shelf-life in Brazil is of about 10 days.

Actions must be taken on improvement of milking
hygienic procedures in order to reduce initial contamination of raw milk in Brazil. A better control of the cold chain is necessary in order to allow the proper exploitation of the
aseptic filling procedures for the pasteurized milk and the extension of the shelf-life.

REFERENCES


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