Microbiological and Sensory Stability of Pasteurized Milk in Brazil

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The purpose of this study was to investigate the influence of storage temperature on the microbiological and sensory stability of homogenized whole pasteurized milk (75 °C/15 s), packaged in high density polyethylene (HDPE) bottle and low density polyethylene (LDPE) pouch, both were monolayer materials pigmented with titanium dioxide (TiO₂). The storage temperatures investigated were 4, 9, and 14 °C. Microbiological evaluation was based on the results of mesophilic and psycrotrophic counts, with 7 log CFU/mL and 6 log CFU/mL, respectively, set as upper limits of acceptability for the keeping quality of milk. Sensory shelf life was determined based on the ratings assigned to the attributes appearance, aroma, flavor and overall appreciation of milk samples (17 ± 1 °C) by 40 panelists using a 7-point hedonic scale (1 = dislike very much, 4 = neither like nor dislike, 7 = like very much). The average scores > 4 and percentage of approval > 50% were used as threshold values for all the attributes assessed. The microbiological shelf life estimated for pasteurized milk packaged in HDPE bottle and stored at 4, 9, and 14 °C were 36, 8, and 5 days, respectively. For milk samples packaged in LDPE pouch, shelf life was estimated at 35, 7, and 3 days, respectively. According to pre set criteria for sensory stability, shelf life for milk in bottle stored at 4, 9, and 14 °C were 28, 9, and 4 days, respectively. For milk in pouch, sensory shelf life obtained was 28, 7, and 2 days, respectively. The results achieved in this study confirmed that exposure to abusive storage temperature (14 °C) dramatically reduced the shelf life of pasteurized milk and that the temperature around 4 °C can be considered ideal for maintaining the microbiological and sensory quality and integrity of the product. Based on the results of this study, HDPE bottle were found to produce better results when compared to LDPE pouch, mainly for abuse storage temperature.

1. Introduction

Because of improper refrigeration, low quality raw material, and an inadequate packaging system, the maintenance of milk quality is a problem in many countries (Rankin, 2002). Raw milk deteriorates in only a few days even when stored under refrigeration temperatures. Moreover, pasteurized (high temperature short time system – HTST) and refrigerated milk has a shelf life of 7 to 28 days (Meunier-Goddik, 2002). Bacterial spoilage is the main limiting factor in extending the shelf life of conventional

Please cite this article as: Petrus R., Loiola C., Silva C. and Oliveira C., (2009), Microbiological and sensory stability of pasteurized milk in brazil, Chemical Engineering Transactions, 17, 939-944 DOI: 10.3303/CET0917157

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 products (Fromm and Boor, 2004). Factors limiting milk stability are well established: bacterial contamination, inadequate packaging system and improper temperature control. Vulnerability of milk's fat and protein to physical-chemical alterations can also lead to deterioration, thus reducing its quality. Cromie (1991) reported the factors that influence the shelf life of pasteurized milk include the quality of the raw material, the binomial temperature/time pasteurization, resistant microorganisms to pasteurization (particularly psycrotrophics), the presence and activity of post pasteurization contaminants, the packaging system and storage temperature post pasteurization which had the greatest impact on the stability of the product. Shelf life of pasteurized milk in Brazil ranges from 3 to 8 days, mainly due to poor cold chain conditions that prevail throughout the country and subject the product to repeated and/or severe temperature abuse. In light of this, this study aimed to evaluate the microbiological and sensory shelf life of pasteurized (75 °C/15 s) homogenized milk filled in high density polyethylene (HDPE) bottle and low density polyethylene (LDPE) pouch at storage temperatures of 4, 9, and 14 °C.

2. Materials and Methods

2.1 Milk samples

Bulked raw milk was heat treated at 75 °C for 15 s in a HTST plate system, homogenized in a one-stage homogenizer (15 MPa) and then cooled to 4 °C. The product was transferred to 1-L HDPE bottle and to 1-L LDPE pouch, both pigmented with titanium dioxide (TiO₂). After processing and packaging, samples were stored in BOD chambers at 4, 9, and 14 °C. All samples were obtained from a local dairy plant in Pirassununga, State of São Paulo/Brazil, immediately after pasteurization.

2.2 Microbial tests

Three pasteurized milk samples in bottle and pouch were analyzed for the growth of aerobic mesophilic and psycrotrophic bacteria. The upper acceptable limit considered for mesophilic and psycrotrophic counts were 7 and 6 log CFU/mL, respectively.

2.3 Acceptability tests

Sensory stability of the milk lots was estimated based on sensory results of a non-trained panel consisted of 40 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) (Stone and Sidel 1993). The tests were accomplished in individual booths lightened with a fluorescent white lamp and the samples were monadically presented, in 50 mL plastic cups labeled with 3 digit code, at a temperature of about 17 °C. Mineral water was provided to cleanse the palate.

2.4 Statistical analysis

The results of the microbiological and sensory analysis were submitted to analysis of variance (ANOVA) and Tukey's test with significance set a priori at P < 0.05, using the statistical software program SAS 9.1.

3. Results and Discussion

3.1 Microbiological stability

Figures 1 and 2 illustrate the development of mesophilic and psycrotrophic bacteria, respectively, over storage time of commercial samples of milk. The analyses, carried out in triplicate, were interrupted when the counts were greater than or equal to 7 and 6 log CFU/mL for mesophilic and psycrotrophic, respectively. Table 1 shows the results of the statistical analysis.

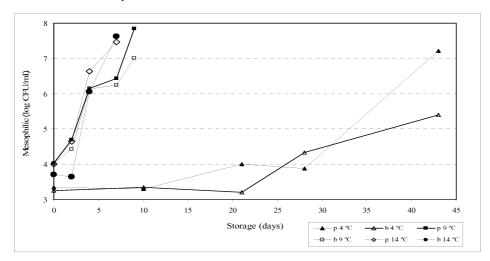


Figure 1-Mesophilic growth in homogenized whole pasteurized (75 °C/15 s) milk filled into HDPE bottle (b) and LDPE pouch (p) at different storage temperatures.

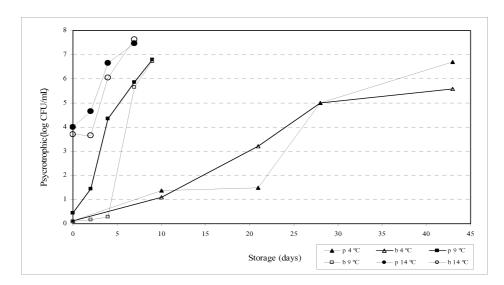


Figure 2-Psycrotrophic growth in homogenized whole pasteurized (75 °C/15 s) milk filled into HDPE bottle (b) and LDPE pouch (p) at different storage temperatures.

Table 1-Statistical results of mesophilic and psycrotrophic counts of homogenized whole pasteurized (75 °C/15 s) milk for different storage times.

Storage temperature	Log CFU/mL ^a									
14 °C	()	2	2 d	4	d	7	' d		
	b	р	b	р	b	р	b	р		
mesophilic	3.7±	4.0±	3.6±	4.6±	6.1±	6.6±	7.6±	7.5±		
	0.1Cb	0.0Da	0.4Cb	0.4Ca	0.2Bb	0.0Ba	0.7Aa	0.1Aa		
psycrotrophic	1.1±	1.1±	3.0±	4.1±	5.5±	6.9±	7.2±	-		
	0.1Da	0.1Ca	0.3Cb	0.3Ba	0.1Bb	0.1Aa	0.9A			
9 ℃		0	2	2 d	4	ł d	7	' d	9	d
	b	р	b	р	b	р	b	р	b	p
mesophilic	4.0±	4.0±	4.4±	4.7±	6.1±	6.2±	6.2±	6.4±	7.0±	7.8±
	0.1Da	0.1Ca	0.1Ca	0.4Ca	0.1Ba	0.2Ba	0.1Bb	0.0Ba	0.0Ab	0.3Aa
psycrotrophic	0.1±	0.5±	0.2±	1.5±	0.3±	4.4±	5.6±	5.9±	6.7±	6.8±
	0.2Ca	0.4Ca	0.2Cb	0.1Ca	0.1Cb	0.3Ba	0.6Ba	0.8Aa	0.0Aa	0.2Aa
4 °C		0	1	0 d	2	1 d	2	8 d	43	3 d
	b	р	b	р	b	р	b	р	b	p
mesophilic	3.3±	3.3±	3.3±	3.2±	4.0±	3.4±	3.9±	5.4±	7.0±	7.2±
	0.1Ba	0.0Ba	0.0Ba	0.3Ba	1.5Ba	0.7Ba	1.5Ba	0.2Ab	0.2Aa	0.2Aa
psycrotrophic	0.1±	0.1±	1.1±	1.4±	3.2±	1.5±	5.0±	5.0±	6.0±	6.7±
	0.1Da	0.0Da	0.2Ca	0.5Ca	0.1Bb	0.4Ca	0.1CA	0.0Ba	0.5Aa	0.7Aa

^aMean values of 3 counts. HDPE bottle (b); LDPE pouch (p); d (days). Means followed by the same lower case letter (comparison between bottle and pouch) or by the same upper case letter (comparison between storage times) within same row are not significantly different (P > 0.05).

3.2 Acceptability test

The average acceptability ratings obtained using the 7-point hedonic scale for each trial, storage at 14, 9, and 4 °C, are shown in Tables 2, 3, and 4, respectively, along with the percentage of consumers that approved the sample by assigning a rating > 4.0. The bold values in all mentioned tables indicate average acceptability ratings ≤ 4.0 and percentage of consumers that approved the sample (by assigning a rating > 4.0) $\le 50\%$.

Table 2-Consumer sensory acceptance of pasteurized milk stored at 14 °C.

Storage time (days)	Attribute	Pouch	Bottle	
		Average ratings	Average ratings	
		/ Approval (%) ^a	/ Approval (%)	
	appearance	5.9 a / 87.5	6.1 a / 95.0	
0	aroma	5.0 a / 60.0	5.4 a / 72.5	
U	flavor	5.0 a / 72.5	5.6 a / 82.5	
	overall appreciation	5.4 a / 77.5	5.7 a / 85.0	
	appearance	5.8 a / 87.5	6.1 a / 97.5	
2	aroma	4.7 a / 52.5	5.3 a / 65.0	
2	flavor	4.1 b / 57.5	5.4 a / 80.0	
	overall appreciation	4.5 c / 55.0	5.6 ab / 87.5	
	appearance	5.7 a / 87.5	5.9 a / 90.0	
4	aroma	4.9 a / 50.0	5.2 a / 65.0	
	flavor	4.5 a / 42.5	5.1 a / 67.5	
	overall appreciation	4.6 a / 50.0	5.3 a / 80.0	

^aPercentage of consumers that assigned ratings of liking > 4.0. Means followed by the same character, within same row for each storage time, are not statistically different (P > 0.05), on a 7-point hedonic scale (1 = dislike very much; 4 = neither like nor dislike; 7 = like very much).

Table 3-Consumer sensory acceptance of pasteurized milk stored at 9 °C.

Storage time (days)	Attribute	Pouch	Bottle	
	_	Average ratings	Average ratings	
		/ Approval (%)	/ Approval (%)	
	appearance	6.1 a / 87.5	6.1 a / 85.0	
0	aroma	5.1 a / 60.0	5.2 a / 60.0	
U	flavor	5.0 a / 67.5	5.2 a / 70.0	
	overall appreciation	5.1 a / 75.0	5.5 a / 77.5	
	appearance	6.1 a / 90.0	5.9 a / 88.0	
7	aroma	5.1 a / 58.0	5.0 a / 60.0	
/	flavor	5.0 a / 65.0	5.1 a / 70.0	
	overall appreciation	5.1 a / 68.0	5.1 a / 68.0	
	appearance	5.6 a / 80.0	5.6 a / 90.0	
9	aroma	4.8 a / 47.5	4.8 a / 52.5	
9	flavor	4.1 b / 42.5	4.4 ab/ 51.0	
	overall appreciation	4.6 b / 52.5	4.9 ab / 62.5	

see Table 2 footnotes.

Table 4-Consumer sensory acceptance of pasteurized milk stored at 4 $^{\circ}$ C.

Storage time (days)	Attribute	Pouch	Bottle	
	_	Average ratings /	Average ratings	
		Approval (%) ^a	/ Approval (%) ^a	
	appearance	6.4 a / 97.5	6.4 a / 95.0	
0	aroma	5.7 a / 77.5	5.3 a / 67.5	
	flavor	5.8 a / 85.0	5.7 a / 80.0	
	overall appreciation	6.0 a / 87.5	5.8 a / 80.0	
28	appearance	6.3 a / 90.0	6.2 a / 90.0	
	aroma	5.5 a / 57.5	5.4 a / 60.0	
	flavor	4.8 a / 52.5	5.6 a / 77.5	
	overall appreciation	5.0 b / 57.5	5.7 a / 80.0	
43	appearance	6.0 a / 82.5	6.1 a / 95.0	
	aroma	4.5 b / 45.0	5.2 a / 62.5	
	flavor	2.9 b / 20.0	4.0 a / 70.0	
	overall appreciation	3.5 b / 27.5	5.4 a / 75.0	

see Table 2 footnotes.

3.3 Milk shelf life

Table 5 shows the estimated shelf life for pasteurized milk according to pre set microbiological and sensory criteria.

Table 5-Estimated shelf life (days) for homogenized whole pasteurized milk.

Storage temperature (°C)	Microbiological criteria ^a		Sensory criteria ^b		
, ,	pouch	bottle	pouch	bottle	
14	3	5	2	4	
9	7	8	7	9	
4	35	36	28	28	

^aUpper limit considered for mesophilic and psycrotrophic counts: 7 and 6 log CFU/mL, respectively.

4. Conclusions

Abusive temperature of 14 °C dramatically reduce the microbiological and sensory shelf life of pasteurized milk. Ideally, pasteurized milk should be stored at a temperature of about 4 °C for maintaining product quality. It was concluded that the HDPE bottle provides higher stability performance than the LDPE pouch, mainly for abuse storage temperature.

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Acknowledgments

The authors gratefully acknowledge the financial support of the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

^bAverage ratings > 4.0 and percentage of approval > 50% for all sensory attributes assessed on a 7-point hedonic scale.