Effect of temperature - time combination and CO_2 addition on shelf life extension of chilled pasteurized cow milk

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Abstract

The main objective of this research was to study the combined effect of temperature –time (TT) combination and carbon dioxide addition on shelf life extension of chilled pasteurised cow milk. Raw cow milk obtained from the university farm was standardized (3.25% fat), homogenized and pasteurised by High Temperature- Short Time (HTST) method using different TT combinations {72 $^0C/15s$ (TT_1), 74.5 $^0C/13s$ (TT_2), 77 $^0C/12s$ (TT_3), 79.5 $^0C/10s$ (TT_4), 82 $^0C/8s$ (TT_5). Pasteurised milk was bottled and chilled at 4 ^0C. Phosphotase test was conducted to detect the efficiency of pasteurisation. In a separate study pasteurised milk was treated with carbon dioxide and sensory threshold was determined using ASTM (American Society for Testing and Materials) E 679 Ascending Concentration Series Method of Limits to select the suitable carbon dioxide level that can be added into milk. Pasteurised bottled milk was treated with 3 levels of carbon dioxide (0, 7 and 15 mM) and chilled at 4 ^0C. Shelf life of pasteurised, carbon dioxide treated milk was determined using microbiological tests.

The best sensory threshold level of carbon dioxide was 15.61mM. The reduction of Viable Plate Count (VPC) during TT_1, TT_2, TT_3, TT_4 and TT_5 combinations were 96.2%, 96.6%, 97.3%, 91.5% and 93.9% respectively. VPC (log cfu/ml) increased with the storage period in almost all the treatment combinations.

The time needed for pasteurised milk to reach the VPC of 5×10^4 cfu/ml (end of shelf life) varied among the treatment combinations. The longest shelf life of 11 days was obtained by TT_3 at 15 mM carbon dioxide level. Addition of carbon dioxide up to 15 mM, increased the shelf life of pasteurised milk treated with TT_1, TT_2, TT_3, TT_4 and TT_5 combinations by 1, 2, 3, 2 and 3 days respectively.

Introduction

Milk is a perfect food and a satisfactory medium for the growth of microbes. Therefore it acts, as a vehicle to transmit infectious diseases. Hence it is very important to provide the consumer with milk that does not contain any harmful organisms. To achieve this target, various means have been used in the past in order to retain the quality of the milk and to improve the storage life. Among them, the application of heat is widely practiced in the milk industry based on its destructive effect on microorganisms. Heat is applied not only to destroy harmful organisms but also to destroy spoilage microbes and thereby increase the shelf life.

Different types of heat treatments are used in the milk processing industry. Among them pasteurization is the commonest heat processing method of milk for safety. Different temperature-time combinations can be applied to get different quality end products in pasteurization. A combination of heat treatment with other processing alternatives will help to extend the shelf life without any detrimental effect on the sensory qualities of the product.

Incorporation of CO_2 can be used to extend the shelf life of pasteurized milk that will be kept under refrigeration (Robert and Torrey, 1988). CO_2 addition process is cheap as well as safe and apparently does not have any negative effects. CO_2 extends the lag phase and generation time of spoilage microorganisms, especially gram negative, psychrotrophic bacteria so that the shelf life of the perishable food is lengthened. This effect has been commercially applied to many refrigerated products as modified atmosphere packaging. The presence of CO_2 inhibits microbial growth, especially at chilled temperatures and it also reduces the pH of the food product as it dissolves readily in the liquid or fat present.

Therefore, the present study was carried out to find out the combined effect of different temperature-time treatments and carbon dioxide addition on the shelf life extension of chilled pasteurized cow milk.

Materials and Methods

Raw milk obtained from Peradeniya university farm was standardized to 3.25 % fat, homogenized in a two-stage homogenizer (at 1500 and 2500 p.s.i), and High Temperature-Short Time (HTST) pasteurized using different TT combinations such as 72 ^{0}C/15 sec (TT_1), 74.5 ^{0}C/13 sec (TT_2), 77 ^{0}C/12 sec (TT_3), 79.5 ^{0}C/10 sec (TT_4), 82 ^{0}C/8 sec (TT_5) at the Dairy Technology laboratory. Pasteurized milk was chilled at 4 ^{0}C after bottling and the phosphatase test was conducted to check the effectiveness of pasteurisation. The milk was then treated with CO_2 using a commercial carbonated soft drink dispensing unit and the sensory threshold was determined using ASTM (American Society for Testing and Materials) E 679 Ascending Concentration Series Method of Limits (Meilgaard, M. et al., 1999) to select the maximum level of CO_2 that can be added into milk. Pasteurized bottled milk was treated with 3 levels of CO_2 (0, 7 and 15 mM), chilled at 4 ^{0}C, analyzed microbiologically using standard microbiological techniques. A day x temperature-time combination x CO_2 (6 x 5 x 3) factorial arrangement of treatments in a Complete Randomized Design with repeated measurers was utilized for the statistical analysis. Data were taken at 6 time periods (day 01, day 06, day 10, day 13, day 17 and day 21 after processing) for the analysis. Differences among treatments were evaluated by analysis of variance. Analyses were performed using SAS computer package. Duncan's Multiple Range Test was applied for multiple comparisons. Shelf life was estimated by fitting the experimental data to a linear regression.

Results and Discussion

1. Determination of sensory threshold level of CO_2 added in to pasteurized milk

After a preliminary study and a series of sensory studies the 'Group Best Estimated Threshold' for added CO_2 was calculated and is shown in Table 1.

Table 1:The best-estimated threshold of CO_2 (ASTM E-679 Ascending Series Concentration Method of Limits)

Panelist number	CO_2 concentration presented (mM)					Best estimated threshold[c] of individuals	$Log_{(10)}$
	7	9	13	16	18.5		
1	0[a]	0	0	0	+[b]	17.2	1.236
2	0	+	0	0	+	17.2	1.236
3	0	0	+	0	+	17.2	1.236
4	0	+	0	+	+	14.42	1.159
5	0	0	+	0	+	17.2	1.236
6	+	+	0	+	+	14.42	1.159
7	0	0	0	0	+	17.2	1.236
8	0	0	0	0	+	17.2	1.236
9	0	0	0	0	+	17.2	1.236
10	0	0	0	+	+	14.42	1.159
11	0	0	0	+	+	14.42	1.159
12	0	0	+	0	+	17.2	1.236
13	0	0	+	+	+	10.82[e]	1.034
14	0	0	0	+	+	14.42	1.159
15	0	0	0	+	+	14.42	1.159
16	0	+	0	0	+	17.2	1.236
17	0	0	0	+	+	14.42	1.159
18	0	0	+	0	+	17.2	1.236
19	0	+	0	0	+	17.2	1.236
20	0	0	0	+	+	14.42	1.159
21	0	0	0	0	+	17.2	1.236
22	+	0	0	+	+	14.42	1.159
23	0	+	0	+	+	14.42	1.159
							Σ27.456
						Group BET[d] 15.61 mM	Average 1.19

[a,b] felt response by panelists as + or 0

[c] geometric mean of highest concentration missed and the next higher concentration (BET)

[d] geometric mean of individual best estimated thresholds

[e] after omitting BET of panelist 13 (since the value do not fall into the normal distribution) the group BET is 15.88 mM

Meilgaard *et al* (1999) stated that the thresholds are the limits of sensory capacities. There are different thresholds: the absolute threshold, the recognition threshold, the difference threshold and the terminal threshold. The absolute threshold (detection threshold) is the lowest stimulus capable of producing a sensation. The recognition threshold is the level of a stimulus at which the specific stimulus can be recognized and identified. The difference threshold is the extent of change in the stimulus necessary to produce a noticeable difference. The terminal threshold is that magnitude of a stimulus above which there is no increase in the perceived intensity of the appropriate quality for that stimulus; above this level, pain often occurs.

In a sensory determination study conducted by Hotchkiss *et al.* (1999), the sample with 18.6 mM dissolved CO_2 was detected in all the trials by all the panelists. In this experiment also the sample having 18.5 mM was detected by all the panelists. According to the same study, the sensory threshold level determined by a trained panel, for CO_2 in pasteurized milk (2 % fat) was >2.8 mM and <9.1 mM. In this current study where an untrained and trained persons were employed, the estimated sensory threshold was slightly higher as shown in Table 1. It was estimated to be 15.61 mM according to ASTM E 679 Ascending Concentration Series Method of Limits (Meilgaard *et al*, 1999). Addition of CO_2 must be carefully controlled in order to determine the sufficient amount required to minimize or retard spoilage to be added without being detectable by consumers. Therefore determination of sensory threshold level of CO_2 added in to pasteurized milk is very important.

2. Effect of TT combination on the reduction of VPC (cfu/ml) of pasteurized milk at 0 level of CO_2.

Raw milk that was used for the pasteurization, had the average VPC of 4.7 x 10^5 cfu/ ml and pasteurized milk had 1.78 x 10^4, 1.58 x 10^4, 1.26 x 10^4, 3.98 x 10^4, 2.88 x 10^4 (cfu/ml) of VPC in TT_1, TT_2, TT_3, TT_4 and TT_5 temperature-time combinations respectively (Table 2). Therefore the reduction of microorganisms due to the pasteurization using TT_1-TT_5 combinations were 96.2 %, 96.6 %, 97.3 %, 91.5 % and 93.9 %, respectively.

Table 2: Reduction of VPC (log cfu/ml) of pasteurized milk at different TT combinations

TT combination	Mean VPC of raw milk (cfu/ml)	Mean VPC of pasteurized milk (cfu/ml)	Reduction of VPC (%)
TT_1 (72 ^{0}C/15 sec.)	4.7 x 10^5	1.78 x 10^4	96.2
TT_2 (74.5 ^{0}C/13 sec.)	4.7 x 10^5	1.58 x 10^4	96.6
TT_3 (77 ^{0}C/12 sec.)	4.7 x 10^5	1.26 x 10^4	97.3
TT_4 (79.5 ^{0}C/10 sec.)	4.7 x 10^5	3.98 x 10^4	91.5
TT_5 (82 ^{0}C/8 sec.)	4.7 x 10^5	2.88 x 10^4	93.9

According to Henderson (1971), when normal milk of good quality is efficiently pasteurized, approximately 1 % of the bacteria may survive. However, in this experiment, microbial survival percentage was slightly higher than the value stated by Henderson. According to Hall and Trout (1968), pasteurization at 71.7 ^{0}C for 15 seconds destroys 90 to 99 % bacteria present in milk depending upon the original bacterial quality of the milk and the types of organisms present. In addition, they stated that efficient pasteurization destroys 99 % of the organisms; yet in some milk the destruction may be as low as 50 %. Those organisms surviving pasteurization are largely thermodurics and, with modern refrigeration, have little influence on the keeping quality. Therefore the microbial destruction levels in the current experiment were acceptable.

3. Effect of dissolved CO_2 and TT combination on mean VPC of pasteurized milk

A significant ($p<0.05$) temperature-time combination x dissolved CO_2 content x storage time interaction was detected for VPC (log cfu/ml) in pasteurized CO_2 added milk.

As shown in Table 3, significant differences of VPC (log cfu/ml) were observed between the TT combinations used at 0 level of CO_2, on day 01 and 13. It was clear that on day 01, TT_1, TT_2 and TT_3 showed the lower counts and TT_4 & TT_5 showed the higher bacterial counts.

TT_4 (79.5 ^{0}C 10 sec.) showed the highest VPC on day 01 at 0 level of CO_2 (significantly different from TT_1, TT_2 and TT_3). According to this figure, it is clear that different TT combinations used in pasteurization of milk gave different quality end products with reference to the VPC. However, at 7 mM CO_2 level VP Counts were not significantly different between the

TT combinations except on day 17, where some differences were observed, while in the case of 15 mM CO_2 level significant differences were observed between the treatments TT_3, TT_4 & TT_5 on day 13 and 17 only.

Table 3: Effect of dissolved CO_2 and TT combination on VPC (log cfu/ml) of pasteurized milk during 21 days of storage at 4^0C

Days of storage	0 mM CO_2				
	TT_1	TT_2	TT_3	TT_4	TT_5
1	4.25^{abD}	4.2^{aD}	4.1^{aD}	4.6^{cD}	4.46^{bcDC}
6	4.3^{abcD}	4.48^{abcDC}	4.53^{bcC}	4.57^{cD}	4.38^{abcD}
10	4.72^{cC}	4.57^{abcDC}	4.54^{abcC}	4.7^{bcD}	4.67^{abcC}
13	5.24^{cdB}	5.5^{dC}	5.18^{cB}	5.13^{cC}	5.4^{cdB}
17	6.29^{dA}	6.36^{dA}	6.33^{dA}	6.13^{cdB}	6.35^{dA}
21	6.29^{aA}	6.45^{aA}	6.49^{aA}	6.41^{aA}	6.49^{aA}

Days of storage	7 mM CO_2				
	TT_1	TT_2	TT_3	TT_4	TT_5
1	4.28^{abD}	4.19^{aD}	4.05^{aE}	4.16^{aD}	4.28^{abC}
6	4.36^{abcD}	4.28^{abDC}	4.4^{abcD}	4.2^{aD}	4.32^{abcC}
10	4.53^{abcD}	4.56^{abcC}	4.35^{aD}	4.62^{abcC}	4.57^{abcC}
13	5.18^{cC}	5.43^{cdB}	5.23^{cdC}	5.25^{cdB}	5.35^{cdB}
17	5.88^{bcB}	6.28^{dA}	5.69^{abB}	6.3^{dA}	6.43^{dA}
21	6.23^{aA}	6.49^{aA}	6.2^{aA}	6.45^{aA}	6.47^{aA}

Days of storage	15 mM CO_2				
	TT_1	TT_2	TT_3	TT_4	TT_5
1	4.28^{abC}	4.05^{aD}	4.08^{aC}	4.05^{aD}	4.05^{aC}
6	4.38^{abcC}	4.3^{abcDC}	4.23^{aC}	4.28^{abCD}	4.26^{abC}
10	4.54^{abcC}	4.55^{abcC}	4.37^{abC}	4.45^{abcC}	4.6^{abcB}
13	5.13^{cB}	5.23^{cdB}	4.18^{aC}	5.18^{cB}	4.76^{bB}
17	5.46^{aB}	5.35^{aB}	5.57^{abB}	6.27^{dA}	6.26^{dA}
21	6.12^{aA}	6.23^{aA}	6.28^{aA}	6.45^{aA}	6.28^{aA}

(n=6)

a, b, c, d, Means within the same row without a common superscript differ significantly ($p<0.05$)

A, B, C, D, E Means within the same column without a common superscript differ significantly ($p<0.05$)

(TT_1=72 ^0C 15 sec., TT_2=74.5 ^0C 13 sec., TT3=77 ^0C12 sec., TT_4=79.5 ^0C10sec., TT_5=82 ^0C 8 sec.)

The results showed that, with increase of CO_2 concentration, VPC decreased in most of the TT combinations. However, on the final day of experimental period (day 21), there were no differences of VPC between TT combinations in each level of dissolved CO_2. According to Table 3, it is clear that the VPC (log cfu/ml) increased with time in all the treatment combinations except TT_5 at 0 level of CO_2 on day 06. Its value changed between 4.05 – 6.49 log cfu/ml within 21 days of storage period in HTST pasteurized milk with added CO_2. Microbes multiplied rapidly and increased their population when they have their basic needs. Growth requirements for bacteria varied from species to species (Zall, 1990). Milk provides a very good substrate for the microbial growth and multiplication.

4. Effect of TT combination and CO_2 addition on shelf life of chilled pasteurised cow milk

Table 4 : Effect of TT combination and CO_2 addition on the day of reaching the maximum allowable VPC

Temperature-time combination	Days needed at CO_2 concentrations of		
	0 Mm	7 mM	15 mM
TT_1 (72 ^{0}C/15 sec.)	7	8	8
TT_2 (74.5 ^{0}C/13 sec.)	7	8	9
TT_3 (77 ^{0}C/12 sec.)	8	9	11
TT_4 (79.5 ^{0}C/10 sec.)	6	8	8
TT_5 (82 ^{0}C/8 sec.)	6	7	9

5x 10^4 cfu/ml of VPC= maximum VPC-SLS (C.S.181:1972)

According to the SLS (C.S.181: 1972) maximum colony count (VPC) allowed for pasteurized milk is 5x10^4 cfu /ml. Therefore, this value was chosen as an indication of shelf life (termination of shelf life at 5x10^4 cfu/ml of pasteurized milk). The time needed for milk to reach VPC of 5x10^4 cfu/ml was estimated by fitting the experimental data to a linear regression. According to Table 4, at 0 level of CO_2 the highest shelf life of 8 days was obtained by 77 ^{0}C/12 sec. TT combination of pasteurization. Addition of CO_2 at concentrations of 7 and 15 mM increased shelf life of pasteurized milk further to 9 days and 11 days respectively. 72 ^{0}C/15 sec and 74.5 ^{0}C/13 sec. TT combinations used pasteurized milk at 0 level of CO_2 would have a shelf life of 7 days. At 7 mM CO_2 level they would have 8 days of shelf life. At 15 mM of CO_2, 72 ^{0}C/15 sec. pasteurized milk would have 8 days and 74.5 ^{0}C/13 sec. pasteurized milk would possess 9 days of shelf life. Lowest shelf life of 6 days at 0 mM of CO_2 was observed in 79.5 ^{0}C/10 sec. and 82 ^{0}C/8 sec. TT combinations. However, at 7 and 15 mM of CO_2 concentrations both showed an extended shelf life. According to Table 4, the highest shelf life of 11 days was observed in 77 ^{0}C/12 sec. TT combination at 15 mM CO_2 concentration. Thus addition of CO_2 concentrations up to 15 mM would increase the shelf life of pasteurized milk by about 1 to 3 days.

Conclusions

Sensory threshold level of CO_2 was 15.61 mM and addition of CO_2 must be carefully controlled to ensure a sufficient amount to retard spoilage is introduced without being detectable by the consumers.

Different TT combinations used had different microbial killing effects and different shelf lives. The reduction of VPC at 72 ^{0}C/15 sec., 74.5 ^{0}C/13 sec., 77 ^{0}C/12 sec., 79.5 ^{0}C/10 sec. and 82 ^{0}C/8 sec. TT combinations were 96.2 %, 96.6 %, 97.3 %, 91.5 % and 93.9 % respectively. Shelf life of pasteurized chilled (4 ^{0}C) milk derived from above TT combinations can be extended by about 1, 2, 3, 2 and 3 days respectively by the addition of CO_2 concentrations up to 15 mM.

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