Kasetsart J. (Nat. Sci.) 38 : 38 - 43 (2004)

Effects of Preservatives on Raw Milk Components Analyzed by Infrared Spectrophotometry

Nitima Chalermsan[1], Pravee Vijchulata[2], Pornsri Chirattanayuth[2]
Sirinporn Sintuwanit[3], Somjit Surapat [4] and Arunee Engkagul[5]

ABSTRACT

The effects of preservatives on milk component analysis using infrared spectrophotometric method were investigated. Raw milk samples from sixty farms were collected. Thirty ml from each milk sample was kept in plastic vial containing either 0.02 % bronopol, 0.40 % potassium dichromate or sodium azide tablet (0.03 %) as preservatives. All milk sub-samples were stored under refrigeration at 4°C then analyzed for milk components at 0, 7, 14, 21 and 28 days of storage. Control samples (without preservative) were analyzed only at 0 day. The means of milk fat, protein, lactose and total solids for the non-preserved milk at 0 day storage were 4.10, 3.00, 4.84 and 12.65 %, respectively. Analysis result of milk components among the control, milk preserved with bronopol and milk preserved with potassium dichromate showed no statistical different at 0 day storage. At this storage day, however, raw milk with sodium azide had lower ($P<0.01$) fat, protein and total solids. Prolonged storage of milk preserved with the three chemicals over 7 days illustrated a potential decline in most components analyzed. Changing pattern of the analysis results of milk preserved with the three respective chemicals at 28 days when compared with those of 0 day storage were -5.39, -4.79 and -8.36 % for fat, -0.30, -1.17 and +0.13 % for protein, -1.22, -0.91 and -0.91 % for lactose, and -2.45, -2.29 and -3.11 % for total solids. It is concluded that bronopol, potassium dichromate and sodium azide are effective preservatives for raw milk component analysis by infrared spectrophotometry. However, commercial sodium azide in the tablet form contains sodium salt, which can depress milk component analysis result. In addition, preserved raw milk samples should be kept under refrigeration at 4°C and analyzed within 7 days.

Key words : bronopol, potassium dichromate, sodium azide, preservatives, milk components, infrared spectrophotometry

INTRODUCTION

Infrared spectrophotometric analysis for raw milk components is gaining popularity in many developing countries including Thailand. Under the dairy small holding situation in these countries, raw milk samples may be kept for prolong period prior to analysis. To prevent component deterioration, preservative with or without refrigeration of raw milk samples is normally

1 Rajamangala Institute of Technology, Phitsanulok Campus, Phitsanulok 65000, Thailand.
2 Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
3 Department of Animal Science, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.
4 Department of Food Science, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.
5 Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

Received date : 30/01/04 Accepted date : 31/03/04

practiced. Several chemicals have been used as preservatives for raw milk component analysis (Dunham and Kroger, 1985; Kroger, 1985; Bachmann, 1990). These include potassium dichromate ($K_2Cr_2O_7$), sodium azide (NaN_3) and bronopol (2-bromo-2-nitro-1, 3 propanediol). In Thailand, depending on the distance and laboratory workload, the preserved raw milk may be kept for one or two weeks before analysis, the widely used preservatives are potassium dichromate and sodium azide. Although potassium dichromate is an effective preservative, it exhibits harmful effects toward handlers and the environment (Kroger, 1985; Monardes *et al.*, 1995). In addition, the hazardous nature of sodium azide was also reported (Kroger, 1985). On the contrary, bronopol, was reported to have no harmful effect (Ardoe, 1982; Dunham and Kroger, 1985). It has been used as a preservative in the cosmetic's industry. Bronopol, thereby, has become the preservative of choice for raw milk in developed countries (Ardoe, 1982; Kroger, 1985; Bertrand, 1996). Since the potential effectiveness of these three chemicals in combination with refrigeration under prolongs storage of raw milk prior to infrared spectrophotometric analysis is limited, the objective of this study was to investigate the situation.

MATERIALS AND METHODS

Raw milk samples

Composite raw milk samples were collected from sixty farms located in central Thailand. Each composite milk sample was collected in a 1,500-ml plastic bottle and kept in an ice box during transportation to laboratory where it was thoroughly mixed and sub sampled into thirty two 30-ml plastic vials for duplicate analysis. Two vials were without preservative while the rest were equally preserved either with 0.02% (wt/vol) bronopol, 0.40% (wt/vol) potassium dichromate (Bachmann, 1990), or one sodium azide tablet (about eight mg sodium azide equivalent to 0.03%, wt/vol). Milk sub-samples were stored under refrigeration at 4°C until analysis.

Chemical analysis

Each preserved milk sub-sample was analyzed in duplicate for fat, protein, lactose and total solids (TS) at 0, 7, 14, 21 and 28 days of storage. The non-preserved milk (control), was analyzed only at 0 day. Analysis of milk components was conducted by Fourier Transform Infrared Spectroscopy, FTIR (MilkoScan FT6000 spectrophotometer), according to the procedures outlined by Foss electric (1999).

Statistical analysis

Data were analyzed under design of Split plot in time using PROC GLM. Preservative types were arranged in randomized complete block for the main-plot with period of milk sample collection as blocking effects. Storage times were analyzed as repeated effects for the sub-plot. The statistical model used was:

$$Y_{ijklm} = \mu + \rho_i + S_{j(i)} + P_k + PS_{jk(i)} + \varepsilon_{ijk} + D_l + DS_{jl(i)} + PD_{kl} + \delta_{ijklm}$$

where Y_{ijklm} was dependent variables, μ was the overall means, ρ_i was the effect of period of milk sample collection as block (i=1,2,..4), $S_{j(i)}$ was the sampling error (j=1,2,..15), P_k was the effect of preservative types (k=1,2,..4), $PS_{jk(i)}$ was the interaction effect between period of collection and preservative types, ε_{ijk} was the main-plot error, D_l was the effect of storage time (l=1,2,..5), PD_{kl} was the interaction between preservative types and storage time, δ_{ijklm} was the sub-plot error.

Statistical differences of milk components among the types of preservatives for each storage interval and those from storage intervals within each preservative were analyzed by Duncan's multiple range test, when significant interactions were found.

RESULTS AND DISCUSSION

The effects of preservatives and storage times on the analysis of milk fat, protein, lactose and total solids are illustrated in Table 1, 2, 3 and 4 respectively. The means of milk fat, protein, lactose and total solids for non preserved milk at 0 day storage were 4.10, 3.00, 4.84 and 12.65 % respectively. Significant interactions ($P<0.01$) between preservatives and storage were observed. At 0 day storage, analysis of milk samples preserved with bronopol and potassium dichromate provided similar ($P> 0.05$) values of fat, protein, lactose and total solids to those of the controls. Similar results were evident in the report of Foltys *et al*. (1994) that milk components did not differ among non-preserved, bronopol and potassium dichromate preserved milk. However, varying results were reported by Coleman and Moss (1989) who observed that potassium dichromate did not affect fat, but decreased protein percentage in milk samples analyzed within 24 hr after collecting. Monardes *et al*. (1995) and Bertrand (1996) reported that milk preserved with potassium dichromate had lower fat and protein percentages than those preserved with bronopol. These discrepancies probably lie on the concentration of the preservatives used. In the studies of Monardes *et al.* (1995) and Bertrand (1996) only 0.1 % potassium dichromate was used.

As for sodium azide, with the exception of lactose, milk samples preserved with this chemical at 0 day storage were 2.27, 0.50, 0.87 % lower ($P<0.01$) for fat, protein and total solids when compared to the controls, respectively. The depression effect at 0 day storage by sodium azide as observed in this study agrees with Biggs *et al*. (1987) who reported that sodium chloride together with certain minerals gave negative effects at the fat and protein channels of the infrared analyzer. Kroger (1985) observed that the use of one tablet sodium azide for preservation of raw milk samples (30 ml) led to a decrease of 0.036 % fat and 0.020 % protein as determined by Foss MilkoScan. In his study, more depression effect was observed with higher chemical usage levels. The origin of such analysis depression was traced to sodium chloride used as filler at 200 mg/tablet. The sodium azide tablet used in this experiment was analyzed to contain 92 % NaCl (100 mg/tablet). Under normal circumstances, the minerals in raw milk are available at low and almost constant concentration and can be accounted for when the instrument is properly calibrated (Andersen *et al.*, 1993). Hence, if sodium azide tablet is the preservative of choice, it is suggested that the depression effect of the analysis results have to be addressed. This can be rectified by calibrating the instrument against known milk components containing the same amount of sodium azide in tablet form as in the preserved samples (Anonymous, 1977 *cited by* Kroger, 1985). The alternative approach is, perhaps, to use NaCl free sodium azide tablet, as recommended by Grace *et al.* (1992).

Prolonged storage of milk preserved with bronopol, potassium dichromate and sodium azide under refrigeration condition illustrated a potential decline in most composition analyzed. Changing pattern of the analysis results of milk preserved with the three respective chemicals at 28 day when compared to those of 0 day storage were -5.39, -4.79 and -8.36 % for fat (Table 1), -0.30, -1.17 and +0.13 % for protein (Table 2), -1.22, -0.91 and -0.91 % for lactose (Table 3), and -2.45, -2.29 and -3.11 % for total solids (Table 4). Prolonged storage of preserved milk would cause a decline of fat from lipolysis and lactose from fermentation. Similar declining trend should be expected for protein. However, with the exception of potassium dichromate, no analysis difference ($P>0.05$) for protein throughout the storage intervals was found in milk preserved with the two remaining preservatives in this study. Milk preserved with bronopol show significant ($P<0.01$) depression of fat, lactose and total solids at 14 day storage

Table 1 Means (±SE) of fat (%) in non-preserved and preserved milk with bronopol, potassium dichromate and sodium azide at different storage intervals.

Storage times (days)	Preservatives			
	Non-preserved	Bronopol	Potassium dichromate	Sodium azide
0	4.101 ± .010[a]	4.097 ± .010 [a x]	4.109 ± .009 [a x]	4.008 ± .010 [b w]
7	_	4.053 ± .010 [a x]	4.053 ± .010 [a x]	3.886 ± .106 [b x]
14	_	3.980 ± .009 [a y]	3.996 ± .010 [a y]	3.796 ± .109 [b xy]
21	_	3.949 ± .010 [a y]	3.948 ± .106 [a yz]	3.750 ± .108 [b yz]
28	_	3.876 ± .010 [a z]	3.912 ± .010 [a z]	3.673 ± .110 [b z]

n = 60

[ab] Means with different superscripts in the same row are different P<0.01) by DMRT.

[wxyz] Means with different superscripts in the same column are different (P<0.01) by DMRT.

Table 2 Means (±SE) of protein (%) in non-preserved and preserved milk with bronopol, potassium dichromate and sodium azide at different storage intervals.

Storage times (days)	Preservatives			
	Non-preserved	Bronopol	Potassium dichromate	Sodium azide
0	3.004 ± .003[a]	3.009 ± .003 [a]	3.000 ± .003 [ab y]	2.989 ± .003 [b]
7	_	3.000 ± .003 [a]	2.993 ± .003 [ab y]	2.983 ± .003 [b]
14	_	2.999 ± .003 [a]	2.963 ± .003 [b z]	2.984 ± .003 [a]
21	_	2.995 ± .003 [a]	2.964 ± .003 [b z]	2.983 ± .003 [a]
28	_	3.000 ± .003 [a]	2.965 ± .003 [b z]	2.993 ± .003 [a]

n = 60

[ab] Means with different superscripts in the same row are different (P<0.01) by DMRT.

[yz] Means with different superscripts in the same column are different (P<0.01) by DMRT.

Table 3 Means (± SE) of lactose (%) in non-preserved and preserved milk with bronopol, potassium dichromate and sodium azide at different storage intervals.

Storage times (days)	Preservatives			
	Non-preserved	Bronopol	Potassium dichromate	Sodium azide
0	4.835 ± .002	4.834 ± .002 [y]	4.840 ± .002 [y]	4.843 ± .002 [x]
7	_	4.829 ± .002 [y]	4.837 ± .002 [y]	4.834 ± .002 [xy]
14	_	4.797 ± .002 [z]	4.815 ± .002 [yz]	4.810 ± .002 [xy]
21	_	4.784 ± .002 [z]	4.802 ± .002 [z]	4.805 ± .002 [yz]
28	_	4.775 ± .002 [z]	4.796 ± .002 [z]	4.799 ± .002 [z]

n = 60

[xyz] Means with different superscripts in the same column are different (p<0.01) by DMRT.

Table 4 Means (± SE) of total solids (%) in non-preserved and preserved milk with bronopol, potassium dichromate and sodium azide at different storage intervals.

Storage times (days)	Preservatives			
	Non-preserved	Bronopol	Potassium dichromate	Sodium azide
0	12.65 ± .11 ^{a}	12.65 ± .11 $^{a\ x}$	12.66 ± .11 $^{a\ x}$	12.54 ± .12 $^{b\ x}$
7	_	12.59 ± .11 $^{a\ x}$	12.58 ± .11 $^{a\ x}$	12.41 ± .12 $^{b\ xy}$
14	_	12.47 ± .11 $^{a\ y}$	12.48 ± .11 $^{a\ y}$	12.29 ± .13 $^{b\ yz}$
21	_	12.42 ± .10 $^{a\ yz}$	12.42 ± .12 $^{a\ yz}$	12.24 ± .12 $^{b\ z}$
28	_	12.34 ± .10 $^{a\ z}$	12.37 ± .11 $^{a\ z}$	12.15 ± .12 $^{b\ z}$

n = 60

^{ab} Means with different superscripts in the same row are different (P<0.01) by DMRT.

^{xyz} Means with different superscripts in the same column are different (P<0.01) by DMRT.

onward. Similarly, milk samples preserved with potassium dichromate obtained a lower (P<0.01) values for fat, protein and total solids at 14 days and for lactose at 21 days storage intervals. As for milk preserved with sodium azide, the analysis of fat, lactose and total solids illustrated significant (P<0.01) depression values at 7, 21, and 14 days storage, respectively.

Variable storage days prior to analysis of preserved raw milk with no component deterioration are evident in the literatures (Coleman and Moss, 1989; Foltys *et al.*, 1994; Monardes *et al.*, 1995; Bertrand, 1996). The essential influencing factors include type and concentration of the preservatives, storage temperature, and initial microbial load of the raw milk. However, with the exception of milk preserved with potassium dichromate, the decline of fat, lactose and total solids in prolonged storage preserved raw milk as observed in this experiment did not follow by an expected decline of protein. In fact, protein content in milk preserved with sodium azide at 28 days storage was slightly higher than the control. Lipolysis of fat and fermentation of lactose in prolonged storage preserved raw milk normally caused a respective increase in free fatty acids and an increment of lactic acid. Both products were reported to enhance the infrared absorption of the protein channel (Biggs *et al.*, 1987; Van de Voort *et al.*, 1987; and Andersen *et al.*, 1993). According to International Dairy Federation (2000) and Biggs *et al.* (1987), infrared signal for protein increased at an average of about 0.01 % for each meqv increase of free fatty acids. On the same token, Hanus *et al.* (1992) observed that 0.01% reduction of lactose in milk from fermentation would accompany by a 'seeming' increase of 0.04% in protein content. This perhaps explains the slight increasing trends of protein in milk preserved with the three chemicals at 28 storage days (Table 2).

CONCLUSION

It is evident from this study that bronopol, potassium dichromate and sodium azide are effective preservatives for raw milk component analysis by infrared spectrophotometry. However, commercial sodium azide in the tablet form may contain sodium salt, which will depress milk component analysis and, therefore, should be avoided. If tablet sodium azide has to be used, the instrument should properly be calibrated. In addition, consideration in selecting the preservative should not only be based on the chemical price but also on its potential environmental and health effects. It is, however, recommended that under

tropical raw milk production situation, the preserved raw milk should be kept under refrigeration at 4°C and analyzed within 7 days.

ACKNOWLEDGEMENTS

The authors would like to thank the National Institute of Animal Health, Department of Livestock Development, Bangkok, Thailand for the assistance and instrumental supports in this study.

LITERATURE CITED

Andersen, T., N. Brems, M.M. Borglum, S. Kold – Christensen, E. Hansen, J.H. Jorgensen and L. Nygaard. 1993. Modern laboratory practice 1: Chemical analysis, pp. 381-416. *In* R.K. Robinson (ed.). **Modern Dairy Technology.** Volume 2: Advances in Milk Products. Elsevier Science Publishers Ltd., London.

Ardoe, Y. 1982. Bronopol as a preservative in milk sample for the determination of cell content using Fossomatic. **Milchwissenschaft** 37(3): 139 – 142.

Bachmann, M.R. 1990. Quality control at reception, pp. 54-87. *In* **Handbook on milk collection in warm developing countries.** International Dairy Federation, special issue No. 9002, 41 Square Vergote, B-1040 Brussels, Belgium.

Bertrand, J.A. 1996. Influence of shipping container, preservative and breed on analysis of milk components of shipped samples. **J. Dairy Sci.** 79: 145-148.

Biggs, D.A., G. Johnsson and L.O. Sjaunja. 1987. Analysis of fat, protein, lactose and total solids by infra-red absorption, pp. 21-29. *In* **Monograph on rapid indirect methods for measurement of the major components of milk.** Bulletin of the International dairy federation. No. 208/1987. Brussels, Belgium.

Coleman, D.A. and B.R. Moss. 1989. Effects of several factors on quantification of fat, protein and somatic cells in milk. **J. Dairy Sci.** 72: 3295-3303.

Dunham, J.R. and M. Kroger. 1985. Milk preservatives. **Dairy herd improvement.** Available Source: http://www.inform.umd.edu/EdRes/Topic/AgrEnv/ndd/dairy/MILK_PRESERVATIVES, May 15, 2001.

Foltys, V., J. Pazmova and E. Bezakova. 1994. Preservative properties of potassium dichromate and bronopol in relation to the milk infrared analysis. **Polnohospodarstvo (Slovak Republic)** 40(2): 144-152. **Food and Human Nutrition Database.** Accession No. 96-139022.

Foss electric. 1999. **CombiFoss 6000 Type 76100 Operator's Manual.** Foss electric, Hillerod. 118 p.

Grace, V., G.A. Houghtby, H. Rudnick, K. Whaley and J. Lindamood. 1992. Sampling dairy and related products, pp. 59-83. *In* R.T. Marshall, ed. **Standard Methods for the Examination of Dairy Products.** 16th ed. The American Public Health Association, Washington, DC.

Hanus, O., V. Gencurova and B. Gabriel. 1992. The effect of aging of milk samples on the accuracy of infrared analysis of milk composition. **Veterinarni-Madicina.** 37(2):149-160.

Kroger, M. 1985. Milk sample preservation. **J. Dairy Sci.** 68: 783 – 787.

International Dairy Federation. 2000. Whole milk: Determination of milk fat, protein and lactose content, guidance on the operation of mid-infrared instruments. **International IDF Standard.** 141C: 2000.

Monardes, H. G. R. K. Moore, B. Corrigan and Y. Rioux. 1995. Preservation and storage mechanisms for raw milk samples for use in milk – recording schemes. **J. Food Protection** 59 (2): 151 – 154.

Van De Voort, F. R., S. Kermasha, J. P. Smith, B. L. Mills and K. F. Ng-Kwai-Hang. 1987. A study of the stability of record of performance milk samples for infrared milk analysis. **J. Dairy Sci.** 70: 1515-1523.