J Food Sci Technol, 2006, 43(4), 425-429

Effect of chemical contaminants on preservation of milk - a comparative study

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Raw liquid milk with specific gravity 1.017±0.010, titratable acidity 0.084±0.008 and pH 6.78±0.063 was treated with different levels of formaldehyde, hydrogen peroxide, boric acid, nisin, oxytetracycline hydrochloride to study their efficacy for its preservation in sterilised glass containers at ambient (27±4°C) and chill (7±2°C) temperatures. The treatments gave varying shelf life, physico-chemical, microbiological and sensory values. Sensory data showed that milk samples were acceptable upto 100, 100, 2500, 100 and 500 ppm levels of formaldehyde, hydrogen peroxide, boric acid, nisin and oxytetracycline hydrochloride, respectively. Treatments with formaldehyde (50 and 100 ppm), hydrogen peroxide (50 and 100 ppm), boric acid (1000 and 2500 ppm), nisin (50 and 100 ppm) and oxytetracycline hydrochloride (50 and 100 ppb) extended the shelf-life of milk from about 7 and 43 h for control to about 46 and 260, 42 and 119, 41 and 241, 20 and 100 and 21 and 100 h at ambient and chill temperatures, respectively.

Keywords: Milk, Preservation, Chemical contaminants, Quality, Stability, Shelf-life

Raw fresh milk has a limited shelf-life of a few hours at ambient conditions due to rapid proliferation of spoilage microflora, activity of endogeneous enzymes and exposure to heat, light and air. The microbial count in milk is a major quality indicator, as most of the microorganisms are undesirable as they can be pathogenic or bring undesirable changes in milk quality. Lowering of temperature retards microbial growth and undesirable changes in milk. In India, a large bulk of milk produced by millions of illiterate people and small farmers, has high microbial loads due to lack of sanitation. Lack of inadequate refrigeration and transportation systems further deteriorates its quality. On the contrary, pasteurisation and ultra high temperature processing enhances keeping quality and extends the shelf-life by partially inhibiting the microorganisms and enzymes. However, the heat treatment causes a number of undesirable changes in nutritional and sensory quality of milk (Fox 1996). Both refrigeration and heat treatment add to the cost of the product. Hence, a number of chemicals have been used singly or in combination to preserve the quality and extend shelf-life of milk (Zapicao et al 1988, Hossain et al 1989, Jandal and Rai 1989, Ambadkar et al 1991, Ambadkar and Lembhe 1994, Abd-El-Hady et al 1995, Foltys et al 1995, Boussouel et al 2000, Saha et al 2003). This study was undertaken to extend the shelf-life of fresh milk under different storage temperature conditions using certain common chemicals and established antibiotics, commonly detected as contaminants in market milk. Effect of these selected chemicals and antibiotics on microbial, chemical and sensory quality parameters was also investigated.

Materials and methods

Fresh untreated milk was procured locally from a single source with minimum time lapse between milking and treatments.

Chemicals used for treatments: Formalin (36% w/v), hydrogen peroxide solution (29%), nisin (Nisaplin with 2.5% activity), boric acid crystals AR (99.5%), oxytetracycline hydrochloride (96.2%) (Riedel-de Haen, Sigma-Aldrich Laborchemikalien GmbH) were added in known quantities to fresh milk samples for preservation.

Treatments: The milk samples having an overall acceptance score of ≥7.5, after giving treatments with no chemical (Control, C), 50 ppm formaldehyde (FD1), 100 ppm formaldehyde (FD2), 50 ppm hydrogen peroxide (HP1), 100 ppm hydrogen peroxide (HP2), 1000 ppm boric acid (BA1), 2500 ppm boric acid (BA2), 50 ppm nisin (N1), 100 ppm nisin (N2), 50 ppb oxytetracycline hydrochloride (OTC1) and 100 ppb oxytetracycline hydrochloride (OTC2), were used in the experiment.

Storage study: Treated as well as control milk samples were stored in sterile glass bottles at ambient (AT) and chill (CT) temperatures in the dark. The stored samples were analysed for chemical, sensory and microbiological quality parameters at a regular interval. Shelf-life of the stored milk was adjudged on the basis of subjective evaluation or coagulation on standing/heating test.

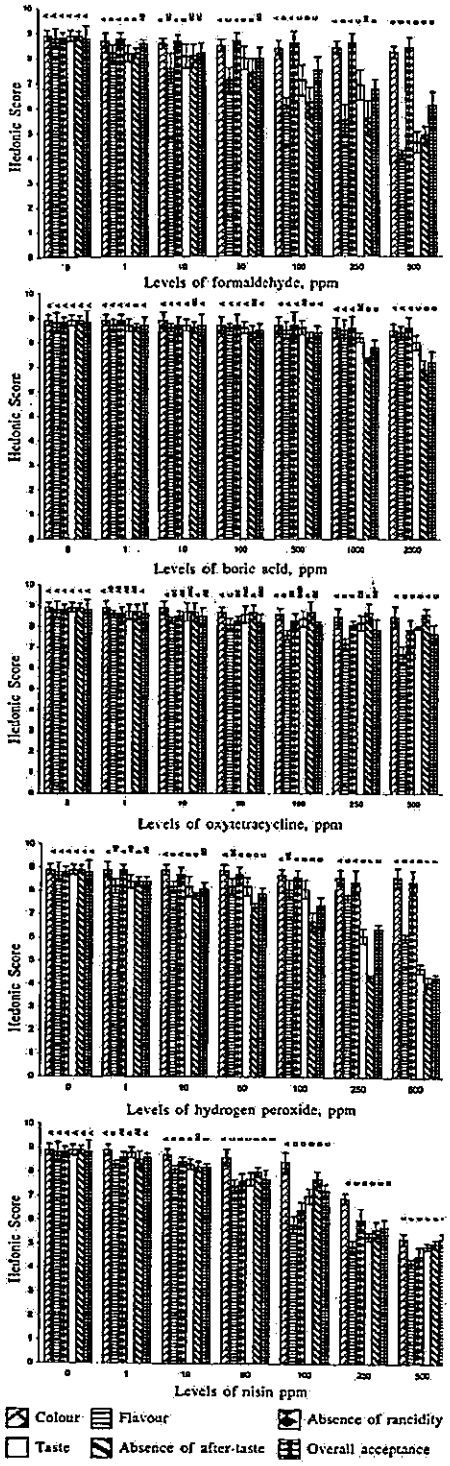
Milk analysis: Standard plate count (SPC), coliforms count (CF), *Staphylococcus aureus* count (SA) were enumerated by the pour plate technique using plate count agar (PCA, HIMEDIA-M091), violet red bile agar (VRBA, HIMEDIA–M049) and Baird Parker agar (BPA, HIMEDIA–M043) media, respectively (APHA 1992, ISI 1977). Specific gravity of milk was measured by using RIMCO lactometer at 60°F (Aggarwal and Sharma 1961). Milk pH was measured using CyberScan pH 2500 Bench meter (Eutech Instruments Pte Ltd, Singapore) and titratable acidity by ISI (1981) method.

Sensory analysis: Raw fresh milk treated with formaldehyde (FD), hydrogen peroxide (HP) and nisin (N) up to 500 ppm, boric acid (BA) up to 2500 ppm and oxytetracycline hydrochloride (OTC) up to 500 ppm was evaluated for sensory attributes like colour, flavour, taste, absence of rancidity, absence of after taste and overall acceptance by a panel of 8 members, using 9-point Hedonic scale (9-like extremely and 1-dislike extremely) (Amerine et al 1965).

Statistical analysis: Data were subjected to analysis of variance (Snedecor and Cochran 1989).

Results and discussion

Sensory analysis: Sensory attributes like flavour, taste and absence of after taste in treated milk were affected signifi-



Capital letters in graphs with different superscripts for each attribute shows significance, (p<0.01)

Fig. 1. Effect of chemicals on sensory attributes of treated milk on 9-point Hedonic scale

Table 1. Effect of storage on quality and stability of untreated control milk

	AT, 0 h	CT, 24 h	CT, 48 h
Acidity, %	0.08±0.008^A	0.14±0.009^B	0.21±0.011^C
pH at 25°C	6.8±0.06^A	6.2±0.05^B	6.0±0.04^C
SPC, log_{10}cfu/g	6.0±4.34^A	6.3±4.25^B	6.5±4.31^C
SA, log_{10}cfu/g	3.1±2.10^A	3.2±1.98^A	3.3±2.10^B
CF, log_{10}cfu/g	3.5±2.16^A	4.4±2.84^B	4.5±3.05^C

Shelf-life was 6.7±0.5 h at AT and 43.0±2.9 h at CT; Mean±SD values with different superscripts in a row differ significantly (p<0.01). SPC: Standard plate count, SA: *Staphylococcus aureus* count, CF: Coliform count, AT: Ambient temperature (27±4°C), CT: Chill temperature(7±2°C); n=8

cantly (p<0.01) by FD treatment at 1 ppm and overall acceptance at 100 ppm levels (Fig. 1). The milk treated with FD even at 1 ppm level was perceived to have presence of added chemicals. Sensory attributes like flavour, taste, absence of after-taste and overall acceptance were affected significantly (p<0.01) by HP treatment at 10, 10, 1 and 10 ppm, respectively (Fig. 1). Lingering tongue biting after-taste was perceived even at 1 ppm of HP in milk. Taste, absence of after-taste and overall acceptance were affected significantly (p<0.01) by BA treatment at 1000, 1 and 1000 ppm levels, respectively (Fig. 1). All the sensory attributes like colour, flavour, absence of rancidity, taste, absence of after-taste and overall acceptance decreased significantly (p<0.01) by N treatment at 250, 1, 10, 10, 10 and 10 ppm levels. (Fig. 1). A change in milk colour to pinkish dull yellow and development of saltiness in taste were noted at ≥100 and ≥10 ppm N levels, respectively. Flavour, absence of rancidity, taste and overall acceptance values decreased significantly (p<0.01) at 10, 50, 100 and 100 ppm of OTC treatment levels (Fig. 1). Overall acceptance score of ≥7.5 was considered for determining acceptable levels for addition of chemicals for milk preservation. Thus, the maximum levels of adding FD, HP, BA and N were considered to be 100, 100, 2500 and 100 ppm, respectively. Although overall acceptance score of 7.7 was noted at 500 ppm of OTC, its maximum level of addition was found to be 100 ppb (the maximum permissible limit in milk).

Shelf-life: Shelf-life of milk for C, FD1, FD2, HP1, HP2, BA1, BA2, N1, N2, OTC1 and OTC2 treatments was 6.7 and 43, 26.7 and 140.7, 46 and 260, 26.3 and 97.3, 42.3 and 119.3, 2 and 144, 4 and 241.3, 15.3 and 69.3, 2 and 100, 14.3 and 70.3 and 21.3 and 100 h at AT and CT, respectively (Tables 1-6). Results showed that the treatments with the selected chemicals increased the shelf-life of milk significantly (p<0.01) at ambient as well as chill temperature.

Titratable acidity: Acidity of untreated control milk (C) increased significantly (p<0.01) after 24 and 48 h storage at CT (Table 1). In FD1 treated milk, there was a significant (p<0.01) increase in acidity after 24 h storage at AT (Table 2). Acidity in HP1 treated milk increased significantly (p<0.01) after 24 and 48 h storage at AT and CT, respectively. In case of HP2 treated milk, a significant (p<0.01) increase in acidity was observed after 48 h storage at AT (Table 3). Acidity in BA treated milk increased significantly (p<0.01) after 48 h storage at CT at 1000 as well as 2500 ppm. There was a significant (p<0.01) increase in acidity in BA2 treated milk after 24 h storage at AT (Table 4). Acidity in N1 and N2 treated milk samples increased significantly (p<0.01) after 24 h storage at CT (Table 5). Milk treated with 50 and 100 ppb OTC showed a significant (p<0.01) increase in acidity at AT on 24 h storage and not at CT even after 48 h storage (Table 6). The milk samples of C, BA1, N1 and N2 after 24 h and HP1, OTC1 and OTC2 after 48 h storage got spoilt or coagulated on standing at AT and hence, were not analysed for acidity.

In general, the titratable acidity showed an increasing trend during storage and significantly (p<0.01) differed from the control in most of the cases. Similar findings were reported for the milk preserved with formalin (Jandal and Rai 1989,

Table 2. Effect of spiking level and storage on quality and stability of formaldehydetreated milk

	AT, 0 h	AT, 24 h	CT, 24 h	AT, 48 h	CT, 48 h
			Formaldehyde, 50 ppm (FD1)		
Acidity, %	0.08±0.006^{A}	0.13±0.009^{D}	0.09±0.003^{A}	0.19±0.013^{C}	0.11±0.010^{AB}
pH at 25°C	6.8±0.02^{A}	6.4±0.02^{C}	6.7±0.02^{A}	6.0±0.04^{D}	6.6±0.04^{B}
SPC, log_{10}cfu/g	6.0±4.22^{AB}	6.0±4.29^{A}	5.9±4.10^{C}	6.0±4.44^{B}	5.8±4.18^{D}
SA, log_{10}cfu/g	2.9±1.95^{A}	2.3±1.65^{B}	2.3±1.52^{B}	2.4±1.68^{B}	2.2±1.38^{B}
CF, log_{10}cfu/g	3.1±2.02^{AB}	3.0±1.90^{A}	3.0±1.87^{A}	3.1±1.87^{B}	3.2±2.00^{B}
			Formaldehyde, 100 ppm (FD2)		
Acidity, %	0.08±0.008^{A}	0.10±0.004^{A}	0.09±0.004^{A}	0.12±0.010^{AB}	0.10±0.008^{AB}
pH at 25°C	6.8±0.04^{A}	6.6±0.02^{B}	6.8±0.01^{A}	6.4±0.02^{C}	6.7±0.02^{A}
SPC, log_{10}cfu/g	5.8±4.28^{E}	5.7±4.02^{E}	5.7±3.98^{F}	5.6±4.04^{F}	5.3±3.94^{G}
SA, log_{10}cfu/g	2.9±1.97^{A}	1.4±1.11^{C}	ND^{D}	ND^{D}	ND^{D}
CF, log_{10}cfu/g	3.0±1.89^{AB}	2.5±1.45^{CD}	2.6±1.38^{C}	1.2±0.0^{E}	2.3±1.56^{D}

Shelf-life: 26.7±2.0 h at AT and 140.7±5.7 h at CT for FD1 and 46±2.2 h at AT and 260±7.5 h at CT for FD2; Mean±SD with different superscripts in a row differ significantly ($p<0.01$); SPC, SA, CF, AT, CT as in Table 1; n=8; ND= Not detectable

Table 3. Effect of spiking level and storage on quality and stability of hydrogen peroxidetreated milk

Parameters	AT, 0 h	AT, 24 h	CT, 24 h	AT, 48 h	CT, 48 h
			Hydrogen peroxide, 50 ppm (HP1)		
Acidity, %	0.09±0.006^{A}	0.14±0.010^{BC}	0.11±0.010^{AB}	Not done	0.17±0.011^{C}
pH at 25°C	6.8±0.02^{A}	6.2±0.01^{D}	6.5±0.02^{C}	Not done	6.1±0.02^{E}
SPC, log_{10}cfu/g	5.6±4.06^{E}	5.4±3.89^{D}	5.4±3.98^{D}	Not done	5.6±4.21^{E}
SA, log_{10}cfu/g	1.4±0.90^{BCD}	1.8±1.04^{D}	1.3±0.48^{BC}	Not done	1.6±1.00^{CD}
CF, log_{10}cfu/g	2.8±1.81^{D}	2.6±1.46^{C}	2.6±1.53^{C}	Not done	2.6±1.34^{C}
			Hydrogen peroxide, 100 ppm (HP2)		
Acidity, %	0.09±0.004^{A}	0.10±0.006^{AB}	0.09±0.005^{A}	0.27±0.010^{D}	0.12±0.008^{AB}
pH at 25°C	6.8±0.03^{A}	6.6±0.02^{B}	6.7±0.01^{A}	5.4±0.02^{F}	6.5±0.01^{C}
SPC, log_{10}cfu/g	5.1±3.86^{BC}	5.0±3.79^{B}	4.9±3.08^{A}	5.4±3.86^{D}	5.1±3.19^{C}
SA, log_{10}cfu/g	ND^{A}	1.8±0.95^{D}	ND^{A}	1.7±0.78^{D}	0.9±0.30^{B}
CF, log_{10}cfu/g	2.4±1.41^{B}	2.1±1.20^{A}	2.3±1.23^{AB}	2.2±1.56^{AB}	2.3±1.41^{AB}

Shelf life: 26.3±2.5 h at AT and 97.3±4.1 h at CT for HP1 and 42.3±1.2 h at AT and 119.3±4.4 h at CT for HP2; Mean±SD values with different superscripts in a row for a parameter differ significantly ($p<0.01$); n=8; SPC, SA, CF, AT, CT as in Table 1; ND= Not detectable

Table 4. Effect of spiking level and storage on quality and stability of boric acid treated milk

	AT, 0 h	AT, 24 h	CT, 24 h	CT, 48 h
		Boric acid, 1000 ppm (BA1)		
Acidity, %	0.17±0.007^{A}	-	0.19±0.009^{A}	0.23±0.008^{B}
pH at 25°C	6.1±0.02^{F}	-	6.1±0.01^{F}	5.9±0.02^{E}
SPC, log_{10}cfu/g	5.3±4.20^{BC}	-	5.4±4.17^{C}	6.2±4.56^{E}
SA, log_{10}cfu/g	3.0±1.83^{DE}	-	2.8±1.35^{C}	2.9±1.32^{D}
CF, log_{10}cfu/g	3.1±1.91^{B}	-	2.9±1.60^{AB}	3.0±1.89^{B}
		Boric acid, 2500 ppm (BA2)		
Acidity, %	0.26±0.008^{BC}	0.38±0.003^{E}	0.29±0.009^{C}	0.32±0.008^{D}
pH at 25°C	5.8±0.03^{D}	5.0±0.02^{A}	5.6±0.02^{C}	5.5±0.02^{B}
SPC, log_{10}cfu/g	5.0±3.98^{A}	5.4±3.88^{BC}	5.3±3.92^{B}	5.9±4.16^{D}
SA, log_{10}cfu/g	2.7±1.49^{AB}	3.0±1.48^{E}	2.6±1.56^{A}	2.7±1.20^{B}
CF, log_{10}cfu/g	2.9±1.19^{A}	3.3±1.73^{C}	2.8±1.68^{A}	2.9±1.61^{AB}

Shelf life: 25±1.4 h at AT and 144±4.9 h at CT for BA1 and 41.0±3.7 h at AT and 241.3±8.2 h at CT for BA2; Mean±SD with different superscripts in a row differ significantly ($p<0.01$); n=8; -: Not done; SPC, SA, CF, AT, CT as in Table 1; Analysis was not done for 48 h AT

Bajaj and Rai 1992), hydrogen peroxide (Kang et al 1983, Gupta et al 1986, Hossain et al 1989, Ambadkar et al 1991, Abd-El-Hady et al 1995) and bronopol (Radha et al 2004). The chemicals prevented bacterial fermentation by inhibiting the growth of acid producing bacteria, and hence, reduced the titratable acidity development in milk on storage. Acidity in milk is an important quality parameter indicating its freshness. Increase in acidity is due to the multiplication of inhabiting microflora and lactic acid production in milk. Acidity beyond 0.17% is considered to be due to lactic acid production by fermentation. Milk with above 0.3 and 0.6% acidity usually coagulates on boiling and standing, respectively.

pH: There was a significant ($p<0.01$)

Table 5. Effect of spiking level and storage on quality and stability of nisin treated milk

	AT, 0 h	CT, 24 h	CT, 48 h
	Nisin, 50 ppm (N1)		
Acidity, %	0.09±0.006^{A}	0.14±0.010^{B}	0.36±0.014^{D}
pH at 25°C	6.8±0.01^{E}	5.9±0.01^{B}	5.0±0.01^{A}
SPC, log_{10}cfu/g	6.0±4.41^{B}	6.0±4.20^{B}	6.5±4.73^{D}
SA, log_{10}cfu/g	3.0±1.57^{D}	3.0±1.61^{CD}	3.2±1.58^{F}
CF, log_{10}cfu/g	3.3±1.86^{B}	3.7±1.78^{D}	3.9±1.76^{E}
	Nisin, 100 ppm (N2)		
Acidity, %	0.09±0.008^{A}	0.13±0.009^{B}	0.24±0.008^{C}
pH at 25°C	6.7±0.02^{E}	6.2±0.01^{D}	5.9±0.01^{C}
SPC, log_{10}cfu/g	6.0±4.25^{B}	5.9±4.11^{A}	6.2±4.32^{C}
SA, log_{10}cfu/g	2.9±1.38^{BC}	2.7±1.32^{A}	3.2±1.71^{E}
CF, log_{10}cfu/g	3.1±1.51^{A}	3.6±1.64^{C}	3.7±1.95^{D}

Shelf life: 15.3±1.2 h at AT and 69.3±2.6 h at CT for N1 and 20±2.8 h at AT and 100±6.0 h at CT for N2; Mean±SD with different superscripts in a row differ significantly (p<0.01); Analysis was not done for 24 and 48 h AT; SPC, SA, CF, AT, CT as in Table 1; n=8

Table 6. Effect of spiking level and storage on quality and stability of oxytetracycline treated milk

	AT, 0 h	AT, 24 h	CT, 24 h	CT, 48 h
	Oxytetracycline, 50 ppb (OTC1)			
Acidity, %	0.08±0.006^{A}	0.30±0.011^{B}	0.09±0.005^{A}	0.11±0.009^{A}
pH at 25°C	6.8±0.02^{F}	5.5±0.01^{A}	6.8±0.01^{EF}	6.6±0.01^{C}
SPC, log_{10}cfu/g	5.2±3.94^{B}	6.4±4.73^{G}	6.0±4.08^{D}	6.1±4.22^{E}
SA, log_{10}cfu/g	2.8±1.43^{B}	3.5±1.91^{F}	2.9±1.30^{C}	3.0±1.44^{D}
CF, log_{10}cfu/g	3.1±1.61^{B}	3.6±2.07^{F}	3.3±1.85^{C}	3.5±1.72^{E}
	Oxytetracycline, 100 ppb (OTC2)			
Acidity, %	0.09±0.008^{A}	0.29±0.010^{B}	0.09±0.007^{A}	0.10±0.006^{A}
pH at 25°C	6.8±0.01^{EF}	5.6±0.02^{B}	6.7±0.01^{E}	6.6±0.02^{D}
SPC, log_{10}cfu/g	5.0±3.60^{A}	6.1±4.45^{F}	5.7±4.29^{C}	6.0±4.41^{D}
SA, log_{10}cfu/g	2.3±1.26^{A}	3.2±1.72^{E}	2.7±1.29^{B}	2.9±1.26^{C}
CF, log_{10}cfu/g	2.9±1.52^{A}	3.4±1.75^{D}	3.0±1.64^{B}	3.3±1.78^{C}

Shelf life: 14.3±2.0 h at AT and 70.3±3.7 h at CT for OTC1 and 21.3±1.9 h at AT and 100±2.8 h at CT for OTC2; Mean±SD with different superscripts in a row differ significantly (p<0.01); n=8; Analysis was not done for 48h AT; SPC, SA, CF, AT, CT as in Table 1

decrease in pH of the untreated control milk after 24 and 48 h storage at CT (Table 1). In case of FD1 treated milk, there was a significant (p<0.01) decrease in pH after 24 and 48 h storage at AT and CT, respectively (Table 2). There was a significant (p<0.01) decrease in pH after 24 h storage at AT as well as CT in HP1 treated milk. In 100 ppm treated milk, a significant (p<0.01) decrease in pH was observed after 24 and 48 h storage at AT and CT, respectively (Table 3). There was a significant (p<0.01) decrease in pH of BA1 treated milk after 24 h storage at CT. A significant (p<0.01) decrease in pH of BA2 treated milk was observed after 24 h storage at AT and CT (Table 4). There was a significant (p<0.01) decrease in pH of both N1 and N2 treated milk samples after 24 h storage at AT and CT (Table 5). Milk samples treated with 50 as well as 100 ppb OTC showed a significant (p<0.01) decrease in pH after 24 and 48 h storage at AT and CT, respectively. The milk samples of C, BA1, N1 and N2 after 24 h as well as HP1, OTC1 and OTC2 after 48 h storage got spoilt or coagulated on standing at AT and hence, were not analysed for pH.

In general, the pH showed a decreasing trend during storage and significantly (p<0.01) differed from the control in most of the cases. Similar findings were reported earlier for the milk preserved by formalin (Jandal and Rai 1989, Bajaj and Rai 1992) and bronopol (Radha et al 2004). It indicated that the chemicals prevented the growth of acid producing bacteria, and hence, restricted the decline in pH of milk on storage.

Microbiological quality: There was a significant (p<0.01) reduction in SPC and CF as a result of various treatments except N1 on SPC. Similarly, there was a significant (p<0.01) reduction in SA due to the treatments other than FD1, BA1 and N1. The stored treated milk samples had significantly (p<0.01) lower counts than the corresponding untreated controls except N1 treated milk after 48 h of storage at CT. SPC and CF of the untreated control increased significantly (p<0.01) after 24 h and SA after 48 h storage at CT (Table 1). In case of FD1 treated milk, SPC and SA decreased significantly (p<0.01) after 24 h storage at CT and both AT and CT, respectively. In case of FD2 treated milk, SPC decreased significantly (p<0.01) after 24 and 48 h storage at CT and AT, respectively. SA and CF decreased significantly (p<0.01) after 24 h storage at either temperatures. No *Staphylococcus aureus* was detected in milk after 24 h storage under CT and 48 h storage at either temperature. Microbial counts except SA and CF at 0 h at AT, were significantly (p<0.01) greater in the FD1 treated milk than the corresponding counts of the FD2 treated one on storage upto 48 h under both temperatures (Table 2). In HP1 treated milk, SPC decreased significantly (p<0.01) after 24 h storage at either temperature condition. Gradual depletion of HP levels on storage was accompanied with microbial cell death surpassing cell growth at the initial stages followed by the reverse at the later stages of storage. CF decreased significantly (p<0.01) after 24 h storage at both the temperatures. In case of HP2 treated milk, SPC decreased significantly (p<0.01) at CT after 24 h storage, but increased significantly (p<0.01) after 48 h storage at AT. Depletion of HP levels on storage could be the reason for this. No *Staphylococcus aureus* was detected in fresh milk (0 h storage), but SA increased significantly (p<0.01) after 24 and 48 h storage at AT and CT, respectively. CF decreased significantly (p<0.01) after 24 h storage at AT only. Microbial counts except SA after 24 h of storage at AT

were significantly ($p<0.01$) higher in HP1 treated milk than the corresponding counts for the HP2 treated samples on storage upto 48 h at both temperatures (Table 3). For BA1 treated milk, SPC increased significantly ($p<0.01$) after 48 h storage at CT. SA decreased significantly ($p<0.01$) after 24 h storage at CT. In case of the BA2 treated milk, SPC increased significantly ($p<0.01$) after 24 h storage at either temperature. SA increased significantly ($p<0.01$) after 24 h storage at AT. CF increased significantly ($p<0.01$) after 24 and 48 h storage under AT and CT conditions, respectively. Microbial counts except CF at CT storage for 24 and 48 h, were significantly ($p<0.01$) greater in the BA1 treated milk than the corresponding counts of the BA2 treated milk on storage upto 48 h at both temperature conditions (Table 4). At both the levels of N treatment, SPC as well as SA increased significantly ($p<0.01$) after 48 h storage at CT. Moreover, SPC as well as SA decreased significantly ($p<0.01$) after 24 h storage at CT for N2. Microbial counts except SPC at 0 h storage were significantly ($p<0.01$) greater in N1 treated milk samples than the corresponding counts of the N2 treated ones on storage upto 48 h at either temperature (Table 5). In OTC1 and OTC2 treated milk, SPC, SA and CF increased significantly ($p<0.01$) after 24 h storage under AT and CT conditions. All the three microbiological counts for the OTC1 treated milk samples were significantly ($p<0.01$) greater than the corresponding counts for the OTC2 treated milk upto 48 h storage at either temperature (Table 6). The microbial analysis was not carried out for the milk samples of C, BA1, N1 and N2 after 24 h as well as BA2 after 48 h storage at AT.

Conclusion

Milk was organoleptically acceptable on treatments with upto 100, 100, 2500, 100 and 500 ppm levels of formaldehyde, hydrogen peroxide, boric acid, nisin and oxytetracycline hydrochloride, respectively. Untreated milk with specific gravity of 1.017, titratable acidity of 0.084 and pH of 6.8 was shelf stable upto 7 and 43 h at ambient and chill temperatures, respectively. Treatments with 100 ppm formaldehyde, 100 ppm hydrogen peroxide, 2500 ppm boric acid, 100 ppm nisin and 100 ppb oxytetracycline hydrochloride extended the shelf-life to 46 and 260, 42 and 119, 41 and 241, 20 and 100 and 21 and 100 h at ambient and chill temperatures, respectively.

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Received 29 July 2005; revised 01 February 2006; accepted 25 February 2006