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Screening of different novel preservatives for milk Preservation by microbial analysis

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Abstract

In this experiment different preservatives like Hydrogen Peroxide, Methyl and propyl paraben, Kathon, Bronopol, Sodium Omadine, Dowicil, Triclosan and Sodium Azide were used for preservation of milk for analytical purpose. The raw milk samples were collected from livestock research centre, NDRI Karnal. Different preservatives were added with different concentration in raw milk samples and stored at 37 °C for 24 hrs. The preservatives were screened on the basis of their germicidal action against various group of microorganism by microbial analysis using pour plate method. The germicidal action of every preservative was analyzed by using Total plate count, Lactic acid bacterial count, Coliform count, Yeast and mould count. As per results, it can be concluded that, the Dowicil (0.02-0.20%), Methyl Paraben (0.05-0.20%), Propyl Paraben (0.05-0.20%), Triclosan (0.07-0.30%) and Hydrogen peroxide (0.05-0.20%) were having poor germicidal action against all groups of microorganisms. While Sodium Omadine (0.02-0.08%), and Sodium Azide (0.02-0.10%) have good germicidal action against coliform and yeast and mould count but having poor germicidal action against Total plate count, Lactic acid bacterial count. Bronopol (0.05-0.10%) and Kathon (0.20-0.40%) showed good germicidal action against all groups of microorganisms.

Keywords: Screening, novel preservatives, microbial analysis

1. Introduction

Now a day's adulteration in milk & milk product is a major problem for dairy industry. So, Government appoints food safety officer for collection of samples from market. At the time of drawing of sample chemical preservative is added, because of perishable nature of milk and milk products. Thus, The preservative should be in a such way that it should not affect the physico-chemical & compositional parameters of milk & able to preserve milk without microbial spoilage. So for that purpose, In present study ten individual preservatives (Kathon, Bronopol, Sodium azide, Sodium omadine, Dowicil, Triclosan, Hydrogen peroxide, Paraben, Mercuric chloride, Potassium dichromate) were selected on the basis of available literature and variable concentration of each preservative was added to raw cow milk, based on their effective concentration reported in literature. From results it was found that Sodium azide (0.1%), sodium omadine (0.08%) and dowicil (0.2%) were good germicidal action against coliform and yeast & mould in milk. Mercuric chloride (0.4%) is effective against yeast & mould and potassium dichromate (0.4%) is effective against coliform in milk. Hydrogen peroxide (0.2%) and triclosan (0.3%) are ineffective against all groups of microorganisms (bacteria, coliform, yeast & mould). Methyl paraben (0.2%) and propyl paraben (0.15 and 0.20%) are slight effective against coliform in milk.

2. Materials and Methods

Collection of sample

Milk samples were collected from livestock research centre National Dairy Research Institute, Karnal.

Analysis

The raw cow milk sample (250 ml) was placed in screw capped wide mouth autoclaved bottles (500 ml capacity). Based on literature survey, Ten preservatives were screened on the basis of available literature to obtain a list of preservatives that possess germicidal action against most of microorganisms.

Variable concentration of each preservative was added to cow milk, based on their effective concentration reported in literature. Milk samples containing variable concentration of different preservatives were stored at 37°C for 24 h and finally screened on the basis of their germicidal action against various group of microorganisms. The germicidal action was elucidated by assessing the milk samples for Total plate count (TPC), Lactic acid bacterial count (LAB), Coliform count, Yeast and mould count (YMC). Control milk samples without preservatives were also analyzed for Total plate count, Lactic acid bacterial count, Coliform count and Yeast and mould count.

The milk samples were examined for microbiological parameters according to the methods described in IS:5401 1969^[1] (Method for detection and estimation of coliform bacteria in foodstuffs), IS:5402 1969^[2] (Method standard plate count bacteria in foodstuffs), IS:5403 1969^[3] (Method for yeast and mold count of foodstuffs). Where Lactic acid bacteria were analyzed by method described by (Abdulla, 2010)^[4].

3. Result & Discussion

The sodium azide, When added with different concentrations (0.02, 0.03, 0.05, 0.06 and 0.1%) in milk sample and kept at 37°C for 24 hrs, It was found that sodium azide was effective against yeast & mould and coliform at all concentrations but ineffective against bacteria.

At different concentration, when Hydrogen peroxide & triclosan was used for milk preservation, No any germicidal effect of both the preservatives on various microorganism (bacteria, yeast and mould and coliform) was observed and showed too numerous to count (TNTC) counts for total plate count, lactic acid bacteria counts coliform and yeast & mould. By addition of different concentration of mercuric chloride (0.10, 0.20, 0.30 and 0.40%) in milk samples & then analyzed for total plate count, coliform count, yeast & mould count and lactic acid bacteria counts. As shown in Table 3, it was found that mercuric chloride used at all concentrations resulted in nil counts for yeast & mould in the third dilution, however the total plate count, lactic acid bacteria counts and coliform counts were too numerous to count (TNTC) in the third dilution. It is evident from the results obtained that mercuric chloride is effective against yeast & mould at all concentrations but ineffective against bacteria and coliform.

Potassium dichromate was found effective against coliform groups of microorganism when added with different concentration of (0.10, 0.20, 0.30 and 0.40%) as showed in table 2, However the total plate count, lactic acid bacteria counts and yeast and mould counts were too numerous to count (TNTC) in the third dilution.

After addition of different concentration of methyl paraben (0.05, 0.10, 0.15 and 0.20%), When milk samples were analysed, As shown in Table 1, 2, 3 & 4, It showed too numerous to count (TNTC) counts for total plate count, lactic acid bacteria counts and yeast & mould in the third dilution. It is evident from the tables 1, 2, 3 & 4 results obtained that methyl paraben effective against coliform bacteria at 0.20% concentration.

Methyl paraben was stronger than Sodium benzoate and the assessment of synergistic effects indicated that a combination of two preservatives may extend the spectrum of preservative system with potentiated antibacterial / antifungal properties rather than when each used alone.

Propyl paraben showed poor germicidal action. After addition of different concentration of propyl paraben (0.05, 0.10, 0.15 and 0.20%), it was found that at all concentrations too numerous to count (TNTC) counts for total plate count, lactic acid bacteria counts and yeast and mould & slight effective against coliform bacteria at concentration 0.15 and 0.20% was observed.

Isomethyl thiazoline group of kathon preservative when used at different concentration, (0.02, 0.03, 0.05, 0.07, 0.10 and 0.40%), nil counts for coliform and yeast & mould in the third dilution observed. The total plate counts & lactic acid bacteria counts were too numerous to count (TNTC) at concentration of $\leq 0.03\%$ & ≤ 0.10 in third dilution respectively. At concentration 0.40%, it was found effective against all groups of microorganism showed nil count.

In case of sodium omadine (0.02, 0.04, 0.06 and 0.08%), As shown in Table 1,2,3 & 4 it was found that sodium omadine used at all concentrations resulted in nil counts for coliform and yeast & mould in the third dilution, however the total plate count and lactic acid bacteria counts were too numerous to count (TNTC) in the third dilution.

At different concentration of dowicil (0.02, 0.08, 0.14 and 0.20%) preserved milk samples, As shown in Table 1,2,3 & 4, it was found that at all concentrations resulted in nil counts for coliform and yeast & mould in the third dilution, however the total plate count and lactic acid bacteria counts were too numerous to count (TNTC) in the third dilution.

After addition of different concentration of bronopol (0.03, 0.05, 0.07 and 0.10%) in milk samples and analyzed for total plate count, coliform count, yeast & mould count and lactic acid bacteria counts. At concentration 0.10%, it was found that, it is effective against bacteria, coliform and yeast & mould counts. According to saito (1973) also concluded the same thing & found bronopol has an antibacterial activity against *Pseudomonas aeruginosa* to the same degree as against other Gram-negative bacteria as well as Gram-positive bacteria and to a lesser extent against yeast & mold unlike various antibacterial agents.

As Table 5 shows consolidated results for effect of different preservatives against different groups of microorganisms. It is evident From Table 5 that sodium azide (0.1%), sodium Omadine (0.08%) and dowicil (0.2%) are effective against coliform and yeast & mould in milk. Mercuric chloride (0.4%) is effective against coliform and potassium dichromate (0.4%) is effective against yeast and mould in milk. Hydrogen peroxide (0.2%) and Triclosan (0.3%) are ineffective against all groups of microorganisms (bacteria, coliform, yeast & mould). Methyl Paraben (0.2%) and propyl paraben (0.15 and 0.20%) are effective against coliform in milk. Kathon (0.4%) and Bronopol (0.10%) were found to be effective against all groups of microorganisms (bacteria, yeast and mould and coliform) in milk.

Table 1: Effect of all preservatives against total plate count groups of microorganisms in milk

Raw milk count(log cfu/ml)	Total plate Counts (log cfu/ml)					
	Sodium azide					
	0.02%	0.03%	0.05%	0.06%	0.10%	
5.89±0.50	TNTC	TNTC	TNTC	TNTC	TNTC	
	Hydrogen peroxide					
	0.05%	0.1%	0.15%	0.2%		
5.96±0.02	TNTC	TNTC	TNTC	TNTC		
	Mercuric Chloride					
	0.1%	0.2%	0.3%	0.4%		
6.12±0.02	TNTC	TNTC	TNTC	TNTC		
	Potassium dichromate					
	0.1%	0.2%	0.3%	0.4%		
6.45±0.43	TNTC	TNTC	TNTC	TNTC		
	Triclosan					
	0.07%	0.1%	0.1%	0.3%		
6.09±0.02	TNTC	TNTC	TNTC	TNTC		
	Methyl & Propyl Parabens					
	0.05%	0.1%	0.15%	0.2%		
6.76±0.02	TNTC	TNTC	TNTC	TNTC		
	Kathon					
	0.02%	0.03%	0.05%	0.07%	0.1%	0.4%
6.54±0.48	TNTC	TNTC	4.86±0.04	4.75±0.06	4.57±0.02	NIL
	Sodium Omadine					
	0.02%	0.04%	0.06%	0.08%		
6.33±0.47	TNTC	TNTC	TNTC	TNTC		
	Dowicil					
	0.02%	0.08%	0.14%	0.2%		
5.72±0.08	TNTC	TNTC	TNTC	TNTC		
	Bronopol					
	0.03%	0.05%	0.07%	0.1%		
5.89±0.17	NIL	NIL	NIL	NIL		

Data are presented as means ± S.E (n=3), TNTC: too numerous to count

Table 2: Effect of all preservatives against coliform groups of microorganisms in milk

Raw milk count (log cfu/ml)	Coliform (log cfu/ml)					
	Sodium azide					
	0.02%	0.03%	0.05%	0.06%	0.10%	
2.51±0.06	NIL	NIL	NIL	NIL	NIL	
	Hydrogen peroxide					
	0.05%	0.1%	0.15%	0.2%		
3.80±0.02	TNTC	TNTC	TNTC	TNTC		
	Mercuric Chloride					
	0.1%	0.2%	0.3%	0.4%		
3.88±0.04	TNTC	TNTC	TNTC	TNTC		
	Potassium dichromate					
	0.1%	0.2%	0.3%	0.4%		
4.06±0.02	NIL	NIL	NIL	NIL		
	Triclosan					
	0.07%	0.1%	0.1%	0.3%		
3.65±0.06	TNTC	TNTC	TNTC	TNTC		
	Methyl & Propyl Parabens					
	0.05%	0.1%	0.15%	0.2%		
3.84±0.01	TNTC	TNTC	TNTC	TNTC		
	Kathon					
	0.02%	0.03%	0.05%	0.07%	0.1%	0.4%
2.89±0.59	NIL	NIL	NIL	NIL	NIL	NIL
	Sodium Omadine					
	0.02%	0.04%	0.06%	0.08%		
2.65±0.03	NIL	NIL	NIL	NIL		
	Dowicil					
	0.02%	0.08%	0.14%	0.2%		
3.57±0.09	NIL	NIL	NIL	NIL		
	Bronopol					
	0.03%	0.05%	0.07%	0.1%		
5.89±0.17	2.94±0.02	2.12±0.58	1.24±0.005	NIL		

Data are presented as means ± S.E (n=3), TNTC: too numerous to count

Table 3: Effect of all preservatives against Yeast & mold groups of microorganisms in milk

Raw milk count (log cfu/ml)	Yeast & mold (log cfu/ml)					
	Sodium azide					
	0.02%	0.03%	0.05%	0.06%	0.10%	
2.97±0.57	NIL	NIL	NIL	NIL	NIL	
Hydrogen peroxide						
	0.05%	0.1%	0.15%	0.2%		
2.72±0.04	TNTC	TNTC	TNTC	TNTC		
Mercuric Chloride						
	0.1%	0.2%	0.3%	0.4%		
3.64±0.05	NIL	NIL	NIL	NIL		
Potassium dichromate						
	0.1%	0.2%	0.3%	0.4%		
3.96±0.04	TNTC	TNTC	TNTC	TNTC		
Triclosan						
	0.07%	0.1%	0.1%	0.3%		
2.78±0.05	TNTC	TNTC	TNTC	TNTC		
Methyl & Propyl Parabens						
	0.05%	0.1%	0.15%	0.2%		
3.71±0.02	TNTC	TNTC	TNTC	TNTC		
Kathon						
	0.02%	0.03%	0.05%	0.07%	0.1%	0.4%
3.28±0.54	NIL	NIL	NIL	NIL	NIL	NIL
Sodium Omadine						
	0.02%	0.04%	0.06%	0.08%		
2.64±0.09	NIL	NIL	NIL	NIL		
Dowicil						
	0.02%	0.08%	0.14%	0.2%		
2.69±0.03	NIL	NIL	NIL	NIL		
Bronopol						
	0.03%	0.05%	0.07%	0.1%		
2.69±0.09	NIL	NIL	NIL	NIL		

Data are presented as means ± S.E (n=3), TNTC: too numerous to count

Table 4: Effect of all preservatives against Lactic Acid Bacterial groups of microorganisms in milk

Raw milk count(log cfu/ml)	Lactic Acid Bacterial (log cfu/ml)					
	Sodium azide					
	0.02%	0.03%	0.05%	0.06%	0.10%	
6.39±0.54	TNTC	TNTC	TNTC	TNTC	TNTC	
Hydrogen peroxide						
	0.05%	0.1%	0.15%	0.2%		
5.79±0.07	TNTC	TNTC	TNTC	TNTC		
Mercuric Chloride						
	0.1%	0.2%	0.3%	0.4%		
6.15±0.04	TNTC	TNTC	TNTC	TNTC		
Potassium dichromate						
	0.1%	0.2%	0.3%	0.4%		
5.69±0.03	TNTC	TNTC	TNTC	TNTC		
Triclosan						
	0.07%	0.1%	0.1%	0.3%		
6.01±0.02	TNTC	TNTC	TNTC	TNTC		
Methyl & Propyl Parabens						
	0.05%	0.1%	0.15%	0.2%		
6.58±0.03	TNTC	TNTC	TNTC	TNTC		
Kathon						
	0.02%	0.03%	0.05%	0.07%	0.1%	0.4%
6.43±0.29	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
Sodium Omadine						
	0.02%	0.04%	0.06%	0.08%		
6.15±0.69	TNTC	TNTC	TNTC	TNTC		
Dowicil						
	0.02%	0.08%	0.14%	0.2%		
5.60±0.11	TNTC	TNTC	TNTC	TNTC		
Bronopol						
	0.03%	0.05%	0.07%	0.1%		
5.70±0.02	NIL	NIL	NIL	NIL		

Particular	Sodium azide (0.10%)	Hydrogen Peroxide (0.20%)	Mercuric chloride (0.40%)	Potassium Dichromate (0.40%)	Triclosan (0.30%)	Methyl paraben (0.20%)	Propyl paraben (0.20%)	Kathon (0.40%)	Sodium Omadine (0.08%)	Dowicil (0.2%)	Bronopol (0.10%)
TPC	-	-	-	-	-	-	-	++	-	-	+++
Coliform count	+++	-	-	+++	-	+	+	+++	+++	+++	++
YMC	+++	-	+++	-	-	-	-	+++	+++	+++	+++
LAB count	-	-	-	-	-	-	-	+	-	-	+++

Very Good = +++, Good = ++, Fair = +, Poor (no effect) = -

LAB=Lactic Acid Bacterial Count, YMC=Yeast and Mould Count, TPC=Total Plate Count

4. Summary & Conclusions

From study, it was found that sodium azide (0.1%), sodium Omadine (0.08%) and dowicil (0.2%) are effective against coliform and yeast & mould in milk. Mercuric chloride (0.4%) is effective against coliform and potassium dichromate (0.4%) is effective against yeast and mould in milk. Hydrogen peroxide (0.2%) and Triclosan (0.3%) are ineffective against all groups of microorganisms (bacteria, coliform, yeast & mould). Methyl Paraben (0.2%) and propyl paraben (0.15 and 0.20%) are effective against coliform in milk. Kathon (0.4%) and Bronopol (0.10%) were found to be effective against all groups of microorganisms (bacteria, yeast and mould and coliform) in milk. So based on the germicidal action of different preservatives against various groups of microorganism (bacteria, coliform and yeast & mould counts), bronopol and kathon preservatives were found suitable for preservation of milk for analytical purpose at 0.1% and 0.4% concentration respectively.

5. Acknowledgement

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