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Influence of chemical preservatives on the quality and composition indices of raw milk samples

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Abstract

The experiment was conducted to assess the feasibility of sodium azide, hydrogen peroxide, bronopol, azidiol, boric acid and potassium sorbate as milk preservatives before estimation of the indicators of the content and quality of raw milk using instrumental methods. The milk samples were collected from fresh bulked milk of the dairy farm in Riga District. Milk samples were stored at 4 °C and 20 °C. Untreated milk samples were considered as control samples and were tested against samples treated with 0.02 % sodium azide, 0.06 % hydrogen peroxide, 0.04 % bronopol, 0.4 % azidiol, 1.0 % boric acid and 0.5 % potassium sorbate. Microbiological and chemical parameters (total bacteria count, fat and protein content, somatic cell count) of raw milk samples were measured in all samples just before preservation and then regularly after specific time interval. The results showed that the most suitable preservatives for storing milk before the estimation of the indicators of the content and quality of raw milk by using instrumental methods are bronopol, sodium azide and azidiol. To ensure stable milk quality starting from the time of sample collection till the analysis, it is advisable to preserve the samples with the above-mentioned compounds and storage at 4 °C not longer than 96 hours.

Key words: chemical preservatives, milk preservation, milk quality, raw milk.

Introduction

The problem of preservation of milk samples is common in many countries because the diagnostic laboratories are generally far away from the dairy farming communities, transport of the samples to the laboratory for diagnosis is inadequate (Dunham, Kroger 1985). These problems are aggravated by the need for facilities to keep the milk cool in order to minimize bacterial proliferation and sample spoilage prior to examination, as they are generally lacking. In such situations, it is necessary that other means of milk sample preservation, for example by use of chemicals, are explored. Recently scientists have used various milk preservatives (hydrogen peroxide, sodium azide, bronopol, potassium dichromate, boric acid, *Milkofix*, azidiol, ortobor acid) to overcome this problem (Ng-Kwai-Hang, Hayes 1982; Hanus et al. 1992a; Hanus et al. 1992b; Heeschen et al. 1994; Saha et al. 2003; FOSS Electric 2005).

Applying instrumental methods in testing raw milk it is allowed to use preservative agents (FOSS Electric 2005). In the literature, it is possible to find various preservatives for each indicator (total bacteria count, fat and protein content, somatic cell count). For the

optimization of instrumental methods and precise estimation of milk content and quality indicators, it is necessary to find a preservative that could be used to estimate all of the indicators, mentioned above from one sample vial.

The aim of the present experiments was to evaluate the efficiency of various chemical preservatives and determine their influence on the quality and composition indices of raw milk samples performed by instrumental methods.

Materials and methods

The experiments were conducted at the testing laboratory of Piensaimnieku Laboratorija Ltd. and at the Institute of Biology, University of Latvia during the spring period 2006.

Whole milk was collected from one farm located in Riga District in Latvia. Milk samples were collected in the morning, 3 to 5 h after milking. Milk was brought to the laboratory on ice and used for experiments within 1 to 2 h of receipt.

Preservation procedures

This study was conducted over a period of three weeks. Each week a five litre cow milk sample was collected from fresh bulked milk of the dairy farm. The collected milk samples after thorough mixing were divided into three equal parts. Of the three parts, one part was kept as untreated milk (fresh) and two portions were treated with 0.02 % sodium azide (NaN_3), 0.06 % hydrogen peroxide (H_2O_2), 0.04 % bronopol ($C_3H_6BrNO_4$), 0.4 % azidiol, 1.0 % boric acid (H_3BO_3) and 0.5 % potassium sorbate ($C_6H_7KO_2$), each week with specific preservative. Plastic containers were used in milk preservation. Each of the three treatments was further divided into two equal portions. One portion from each treatment was stored at 4 °C and the other portion at 20 °C. The untreated milk samples (control samples) were analyzed immediately after delivery to the laboratory and then after 24 h, as the standard LVS 175:1999 requires raw milk without preservation can be used for analysis only within 36 h. Analysis of preservative-treated milk samples was conducted after 24, 48, 72 and 96 h, respectively. All parameters used to monitor the quality of milk were determined in ten replications.

Microbiological analyses

Somatic cell counts were performed using fluorescent opto-electronic method on a Fossomatic™ FC configured as a CombiFoss™ 6000FC together with a MilkoScan™ FT6000 (FOSS Electric A/S, Denmark) according to LVS EN ISO 13366-3:1997 and manufacturer's recommended procedures (FOSS Electric 2005). Total bacteria counts were determined using flow cytometry method on a BactoScan™ FC analyzer (FOSS Electric A/S, Denmark) and manufacturer's recommended procedures (FOSS Electric 2001a; FOSS Electric 2001b).

Chemical analyses

Fat and protein tests were made using infrared spectroscopy on a MilkoScan™ FT6000 configured as a CombiFoss™ 6000FC together with a Fossomatic™ FC (FOSS Electric A/S, Denmark) according to ISO 9622:1999(E) and manufacturer's recommended procedures (FOSS Electric 2005).

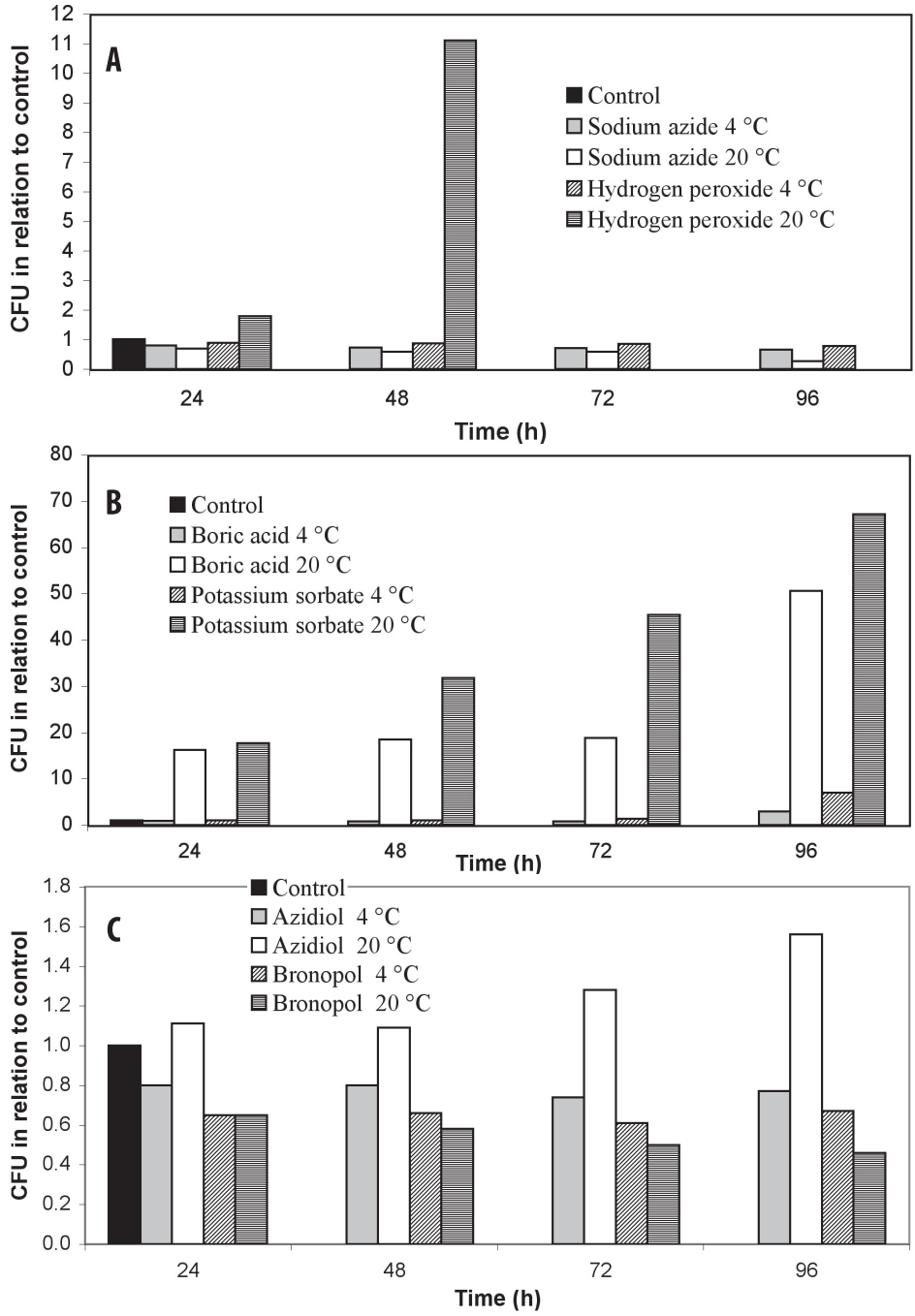

Fig. 1. Total bacteria count (CFU) in the samples of raw milk in relation to chemical preservative treatment estimated using flow cytometry approach. A, 0.02 % NaN_3, 0.06 % H_2O_2. Control value was 397×10^3 CFU ml^{-1}. B, 1.0 % H_3BO_3, 0.5 % $C_6H_7KO_2$. Control value was 250×10^3 CFU ml^{-1}. C, 0.4 % azidiol, 0.04 % $C_3H_6BrNO_4$. Control value was 324×10^3 CFU ml^{-1}. Control samples were analyzed immediately after transport to the laboratory.

Statistical analyses

Data were analyzed using Microsoft Excel and FOSS Electric software – System 4000 version 4.1.8. and FOSS Integrator version 1.3.7. For each count series the average value and standard error (SE) was calculated. The upper and lower 99 % confidence limits (α = 0.01) were estimated.

Results and discussion

Tests with non-preserved milk samples stored at 4 °C for 24 h and 48 h were performed using flow cytometry (FCM). The results indicated differences in ratio of individual bacteria count (IBC) and colony forming units (CFU) depending on the period of sample storage (i.e. 24 h or 48 h). The IBC in the samples after 48-h storage was three to seven times higher, compared to the samples stored for 24 h (Sešķēna, unpublished data). After 24-h storage at 4 °C, the IBC number in the samples increased 1.5 to four times. These results confirm the necessity to develop a preservation approach for infected milk samples with the aim to prolong the quality control period and to provide method precision, because it is impossible to calibrate equipment de novo for every samples series.

The effect of various preservatives was evaluated using criteria of milk content and quality such as the total bacteria count, somatic cell count, and fat and protein content.

The influence of storage temperature on the preservation efficiency was investigated by comparing the quality of raw milk samples stored at refrigeration temperature (4 °C) and ambient temperature (20 °C). The control samples (without addition of preservative), stored at 20 °C for 24 h was fermented and could not be used for analysis. The mean bacteria counts in control samples were higher than 1×10^7 CFU ml^{-1}. For this reason, control samples were tested at time of samples were delivery to the laboratory. Raw milk samples containing preservative were analyzed after 24-, 48-, 72- and 96-h storage. An exception was for samples with H_2O_2 stored at 20 °C, which were tested after 24- and 48-h storage only, because during longer periods total bacteria count exceeded 8×10^6 CFU ml^{-1} and milk was fermented. Thus, a lower stability of hydrogen peroxide at 20 °C is indicated.

The effect of the various preservatives on total bacteria count estimated using flow cytometry in raw milk samples is shown in Fig. 1. Bacteria proliferation during the 96-h period was inhibited in the raw milk samples amended with NaN_3, H_2O_2, $C_3H_6BrNO_4$ and azidiol, when stored at 4 °C. Bacteriostatic effect of H_3BO_3 and $C_6H_7KO_2$ was observed during 72-h storage at 4 °C. In all tested samples, variability of total bacteria count measured by FCM, did not exceeded confidence interval (± 49.02 %, α = 0.01), corresponding to the precision of microbiological methods and not considered as significant.

The antiseptic properties of H_2O_2, H_3BO_3 and $C_6H_7KO_2$ were lost at 20 °C as the total bacteria count was considerably higher already after 24 h storage: 1.8, 16.2 and 17.7 times, correspondingly.

The obtained results indicate a strong dependence of preservative efficiency on the storage conditions, i.e. temperature. It was concluded that the tested preservatives at 4 °C retain their antiseptic properties for a longer period as compared to 20 °C. The most important factors reported to influenc efficiency of preservatives include an initial microbial count in the product, microbial species, temperature and pH of environment (Baltess 1998). In our study, temperature conditions had a the strong effect on preservative

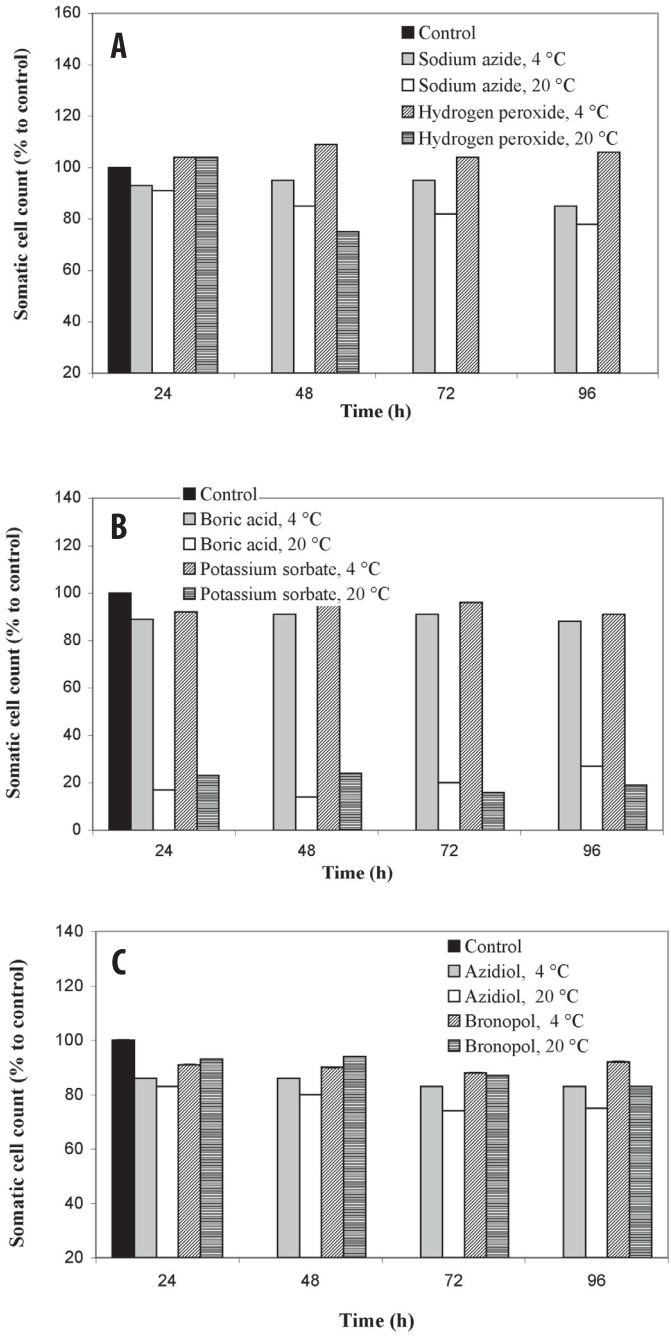

Fig. 2. Somatic cell count in the samples of raw milk in relation to chemical preservative treatment estimated using fluorescent optoelectronic method. A, 0.02 % NaN_3, 0.06 % H_2O_2. B, 1.0 % H_3BO_3, 0.5 % $C_6H_7KO_2$. C, 0.4 % azidiol, 0.04 % $C_3H_6BrNO_4$. Control values were 138 × 10^3 ml^{-1} (A), 212 × 10^3 ml^{-1} (B), 159 × 10^3 ml^{-1} (C). Control samples were analyzed immediately after transport to the laboratory.

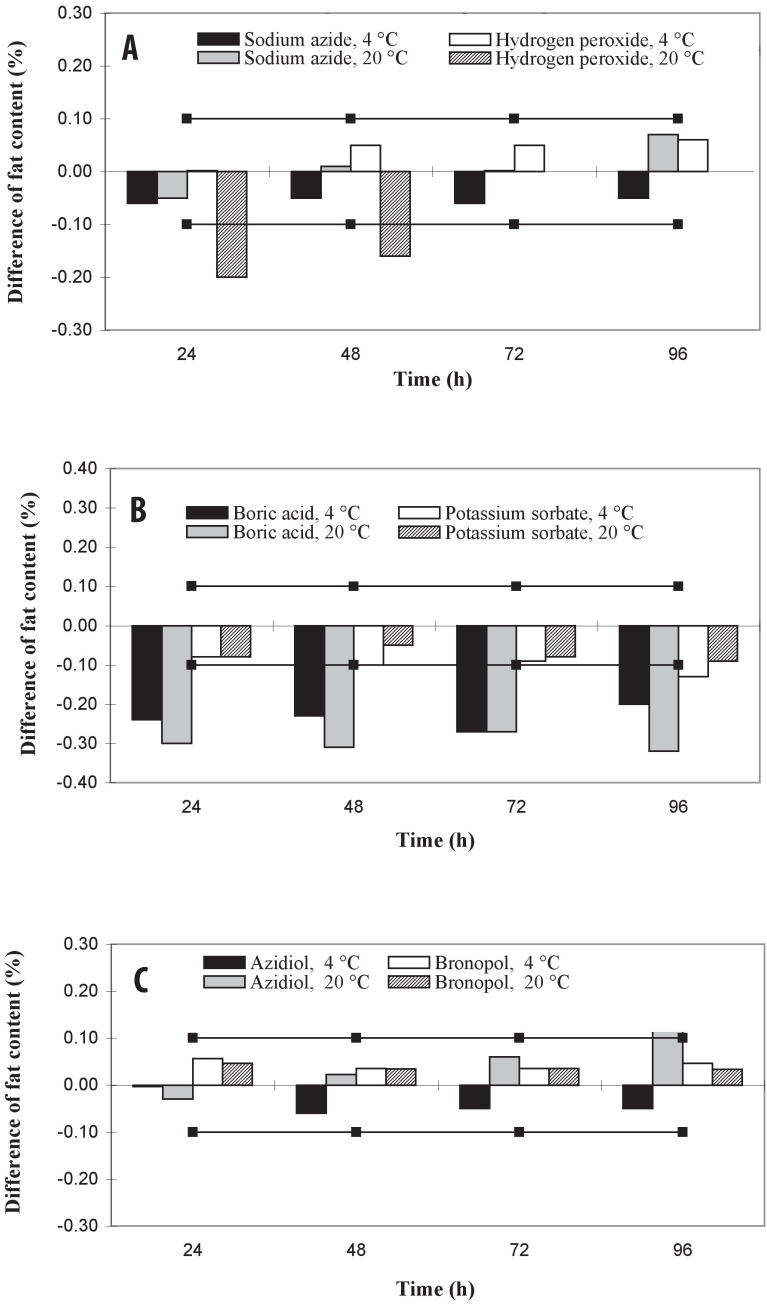

Fig. 3. Fat content in the samples of raw milk in relation to chemical preservative treatment estimated using infrared analysis. A, 0.02 % NaN_3, 0.06 % H_2O_2. B, 1.0 % H_3BO_3, 0.5 % $C_6H_7KO_2$. C, 0.4 % azidiol, 0.04 % $C_3H_6BrNO_4$. Control samples were analyzed immediately after being transported to the laboratory. Control values were 5.84 % (A), 5.14 % (B), 5.78 % (C). Horizontal line represents 99 % confidence interval ($\alpha = 0.01$) of characterized control samples.

efficiency, especially this effect was shown for H_3BO_3, $C_6H_7KO_2$, and H_2O_2.

The influence of NaN_3, H_2O_2, $C_3H_6BrNO_4$, H_3BO_3, $C_6H_7KO_2$ and azidiol on somatic cell count was studied using fluorescent optoelectronic method (Fig. 2). Using these preservatives, the somatic cell count did not change significantly during 96-h storage at 4 °C and did not exceeded confidence interval (± 21.6 %, $\alpha = 0.01$). Our results differ from those obtained by Heeschen et al. (1994), who found that milk preservation with NaN_3 caused significantly lower somatic cell counts. We observed that the somatic cell count in the samples treated with H_3BO_3 and $C_6H_7KO_2$ and stored at 20 °C greatly decreased, i.e. 5.2 and 4.8 times, correspondingly. A decrease of the somatic cell count was detected during the entire period of the experiment. This effect can be explained by decreased cell envelope permeability of somatic cells caused by preservatives at 20 °C, which leads to poor nuclear DNA staining and weak fluorescent optoelectronic detection.

The effect of preservatives on the fat content of raw milk using infrared spectroscopy method is shown in Fig. 3. There were no any considerable changes in fat content in the samples treated with NaN_3, $C_3H_6BrNO_4$, $C_6H_7KO_2$, and azidiol at 4 °C and 20 °C and H_2O_2 at 4 °C during the 72-h period. We observed a significant decrease of fat content after 24 h and 48 h in samples preserved with H_2O_2 and stored at 20 °C, and after 72 h these samples spoiled. The most considerable changes in the fat content were detected in the samples amended with H_3BO_3: fat content decreased up to 0.31 % of the control sample. This effect can be explained by the reaction between H_3BO_3 and CH-groups of lipid molecules, resulted in their altered properties. CH-groups do not absorb light with the wavelength 3.5 μm used for fat content detection by MilkoScan™ FT6000.

The effect of preservatives on the protein content in raw milk using infrared spectroscopy method is shown in Fig. 4. Azidiol, $C_3H_6BrNO_4$, NaN_3 and H_2O_2 did not noticeably affect the milk protein content. The protein content in the samples treated with H_3BO_3, and stored at 4 °C and 20 °C for 96 h, decreased by 0.74 % and 0.87 %, correspondingly. This can be explained by the ability of H_3BO_3 to bind not only with CH-groups of lipid molecules, but also with N-H groups of peptide bounds, thus influencing absorption intensity. H_3BO_3 thus affects the estimation of fat and protein content in raw milk obtained by infrared spectroscopy. The effect of $C_6H_7KO_2$ on protein content measurement was the reverse, i.e. protein content in tested samples stored at 4 °C and 20 °C increased by 0.20 % and 0.39 %, correspondingly. Most probably, the ability of some sites in the potassium sorbate molecule to absorb light at wavelength 6.5 μm interferes with peptide N-H sites, which are known to absorb light at the same wavelength. The literature data indicates light absorption at 6.5 μm also for citric acid [ISO 9622:1999(E)].

The results obtained in this study show that the use of bronopol, sodium azide or azidiol for raw milk preservation could provide stable milk quality and rational use of up-to-date equipment in cases when the samples were immediately refrigerated and stored within the period of 96 h. Similar types of results are obtained by FOSS Electric (2001a; 2005) and Gonzalo et al. (2004) who observed that azidiol and NaN_3 can be used successfully in preserving milk samples for bacteriological analysis on a BactoScan™ FC analyzer, and bronopol is the optimal preservative of milk samples for the CombiFoss™ 6000FC method.

Evaluation of the compounds mentioned above ($C_3H_6BrNO_4$, NaN_3 and azidiol) from the commercial point of view, suggest bronopol as the most appropriate preservative that is commercially available in tablet form. The use of this preservative does not require

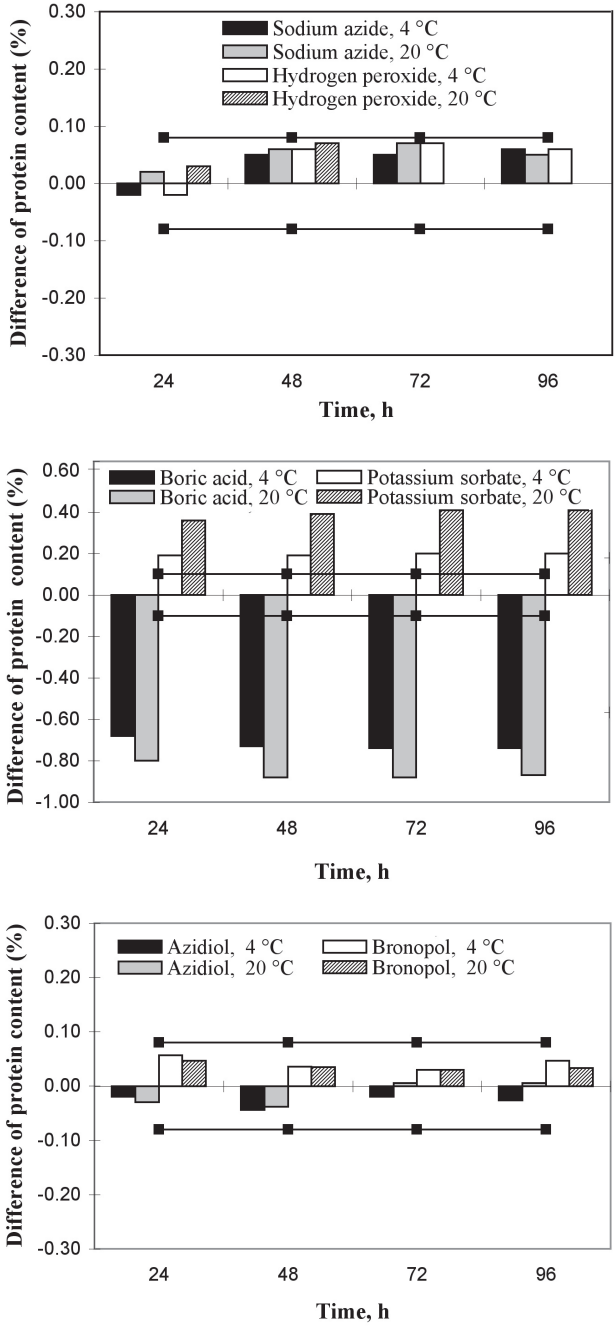

Fig. 4. Protein content in the samples of raw milk in relation to chemical preservative treatment estimated using infrared analysis. A, 0.02 % NaN_3, 0.06 % H_2O_2. B, 1.0 % H_3BO_3, 0.5 % $C_6H_7KO_2$. C, 0.4 % azidiol, 0.04 % $C_3H_6BrNO_4$. Control samples were analyzed immediately after being transported to the laboratory. Control values were 4.21 % (A), 4.86 % (B), 4.34 % (C). Horizontal line represents 99 % confidence interval ($\alpha = 0.01$) of characterized control samples.

additional time for unit-dose packaging. Sodium azide and azidiol are known to be toxic and these compounds do not degrade in the environment, therefore these preservatives are not offered to consumers. These chemicals can be used only for laboratory analyses. However to avoid the potential risk for laboratory personnel and contamination of environment it is necessary to search for new, less harmful agents for raw milk preservation.

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Ķīmisko konservantu ietekme uz koppiena paraugu kvalitātes un sastāva rādītājiem

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Kopsavilkums

Eksperiments veikts, lai noskaidrotu un izvērtētu nātrija azīda, ūdeņraža peroksīda, bronopola, azidiola, borskābes un kālija sorbāta ietekmi uz koppiena paraugu sastāva un kvalitātes rādītājiem, kas noteikti, pielietojot testēšanā instrumentālās metodes. Analīzēm izmantots koppiens, kas iegūts Rīgas rajonā esošā zemnieku saimniecībā. Piena paraugi uzglabāti 4 °C vai 20 °C temperatūrā. Ar 0,02 % nātrija azīdu, 0,06 % ūdeņraža peroksīdu, 0,04 % bronopolu, 0,4 % azidiolu, 1,0 % borskābi un 0,5 % kālija sorbātu konservēti paraugi analizēti paralēli nekonservētiem koppiena paraugiem, kuri izmantoti kontrolei. Kontroles paraugiem mikrobioloģiskie un ķīmiskie rādītāji (baktēriju kopskaits, tauku un olbaltumvielu saturs, somatisko šūnu skaits) noteikti tūlīt pēc koppiena piegādes laboratorijā, bet konservētiem paraugiem – pēc 24, 48, 72 un 96 stundām. Noskaidrots, ka vispiemērotākie konservanti koppiena paraugu kvalitātes un sastāva rādītāju noteikšanai ar instrumentālām metodēm ir bronopols, nātrija azīds un azidiols. Lai nodrošinātu nemainīgu piena kvalitāti no paraugu noņemšanas brīža līdz testēšanai, koppiena paraugus ieteicams konservēt ar šiem savienojumiem un uzglabāt 4 °C temperatūrā ne ilgāk par 96 stundām.