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The Effect of Bronopol on the Freezing Point and Impedance of Milk Samples

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ABSTRACT

Preferred sample-handling guidelines for the testing of raw milk samples cryoscopically require refrigeration followed by analysis within 24 hours. When this practice cannot be followed, the use of a broad-spectrum preservative, such as bronopol, is an acceptable alternative. Cryoscopy's primary function is to detect added water in milk by measuring its freezing point.

This study was designed to quantify the effect of adding a preservative to a milk sample that will be tested cryoscopically. Using raw milk, pasteurized whole milk, and reagent grade water, 11 different concentrations of bronopol were tested 5 times each on a cryoscope, and these results were compared to the baseline readings of the unadulterated samples. The results show a direct, measurable correlation between bronopol concentration and freezing point depression of the samples. When used at the manufacturer's suggested concentration of approximately 0.44 mg/mL, bronopol depresses the freezing point by 10 – 20 m°H.

Impedance testing is used in the dairy industry to estimate shelf life, and as a secondary method to detect adulterated milk products. Impedance methods detect the conversion of nonelectrolytic compounds in milk (i.e., sugars) into ionic compounds (i.e., lactic acid) as a byproduct of microbial metabolic activity. Using an impedance technique to measure the resistance values of the same samples tested on the cryoscope, it was shown that as the bronopol concentration increased from 0 to 1.62 mg/mL, the ohms reading decreased from 1803 to 369. The study clearly demonstrates the quantitative effects of bronopol on freezing point and impedance measurements of milk.

INTRODUCTION

ISO 5764 is an International Standard that specifies the use of a thermistor cryoscope, a widely-accepted reference method, for the determination of the freezing point of raw, pasteurized, UHT-treated or sterilized whole milk, partially skimmed milk, and skimmed milk. Freezing point measurements are used to estimate the proportion of extraneous water in milk. The standard indicates that samples "may be stored at a temperature of between 0°C and 6°C if necessary" and "it is preferable to test the samples immediately upon arrival at the laboratory."

According to the International Standard ISO 707, guidance on sampling milk and milk products, the use of preservatives is permitted for samples of non-sterilized milk and liquid milk products that are going to undergo physical or chemical analysis. This is also true for sterilized milk products if the samples are gathered on the production line or from one or more of the original packages. In some cases, a broad-spectrum preservative such as bronopol is commonly used in combination with refrigeration to stabilize the samples if analysis cannot be performed promptly. Samples should be dispatched immediately and tested within 24 hours. In cases where the preservative interferes with the analyte being tested, a suitable correction factor must be used.

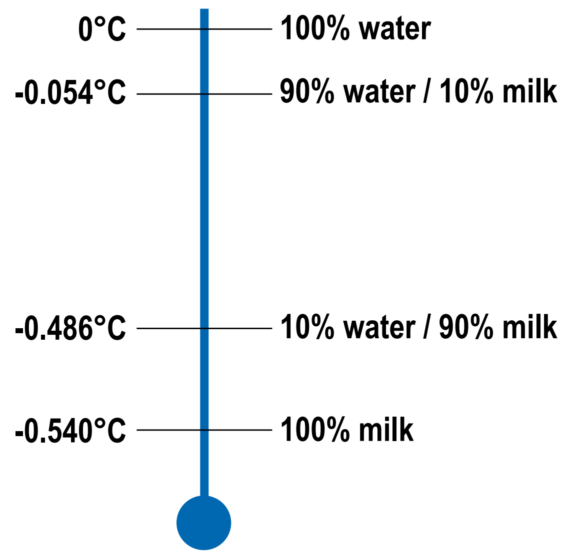

Fig. 1. Linear relationship of freezing water in milk

Pure water freezes at 0°C and pure milk freezes at (hypothetically) –0.540°H. Assuming a linear relationship, each 1% of extraneous water will move the freezing point 1/100th of the difference (0.0054°H) closer to 0°C (Fig. 1). When solutes are added to milk, the freezing point is depressed. Conversely, when water is added to milk, the freezing point is elevated, generating values closer to 0°C. This study was designed to quantify the effect of adding preservative to a milk sample tested cryoscopically to determine its freezing point.

MATERIALS AND METHODS

Sample Preparation

Reagent grade water, pasteurized milk, and raw milk were dispensed by weight into 14, 100 mL glass jars (Table 1). The corresponding amount of broad-spectrum microtab preservative, bronopol (D & F Control Systems, Inc.) was added to each jar and mixed by inversion until the bronopol dissolved. These samples were used for the cryoscopy and impedance portions of the study.

Testing Procedures

Following manufacturer's instructions, a model 4D2 cryoscope (Advanced Instruments, Inc.) was used to test each sample five times. Average values were calculated, and changes in m°H from baseline were reported graphically. Following manufacturer's instructions, the BacTrac (Advanced Instruments, Inc.) impedance instrument was used to test bronopol samples in water. Ohms values (MO) were recorded for each sample. Microsoft® Excel 2000 was used for statistical analyses.

Sample	Bronopol (mg)	Water (mL)	Raw Milk (mL)	Pasteurized Milk (mL)	Bronopol Concentration (mg/mL)
1	0	10.35	10.35	10.35	0.00 mg/mL
2	8.4	10.35	10.35	10.35	0.81 mg/mL
3	8.4	14.79	14.79	14.79	0.57 mg/mL
4	8.4	19.22	19.22	19.22	0.44 mg/mL
5	8.4	23.66	23.66	23.66	0.35 mg/mL
6	16.8	10.35	10.35	10.35	1.62 mg/mL
7	16.8	14.79	14.79	14.79	1.14 mg/mL
8	16.8	19.22	19.22	19.22	0.87 mg/mL
9	16.8	23.66	23.66	23.66	0.71 mg/mL
10	16.8	29.57	29.57	29.57	0.57 mg/mL
11	16.8	38.44	38.44	38.44	0.44 mg/mL
12	16.8	44.36	44.36	44.36	0.38 mg/mL
13	16.8	50.27	50.27	50.27	0.33 mg/mL
14	16.8	59.15	59.15	59.15	0.28 mg/mL

Table 1. Sample preparation

RESULTS

The results of this study showed that when used within the manufacturer's specifications of one tablet per 40 mL milk sample, the broad-spectrum microtab (bronopol) demonstrates a measurable effect on the sample's cryoscopic result (approximate change of 10 to 20 m°H). There is a linear relationship between the bronopol concentration in water, pasteurized milk and raw milk, and the cryoscopic value obtained during testing (Figs. 2 – 4). There is an inverse relationship between the bronopol concentration and the ohms readings taken from the BacTrac (Fig. 5). As the concentration of bronopol increased, the ohms readings (impedance) decreased.

The larger than expected variation in values obtained for the different testing matrices (raw milk, reagent grade water, and pasteurized milk) lead to an evaluation of the uniformity of bronopol tablets. Ten tablets were pulled at random from the dispenser and weighed (Table 2). The weights ranged from 14.7 mg to 19.2 mg, with an average weight of 16.8 mg and a standard deviation of 1.5 mg. These tablet weight discrepancies may explain the anomalies seen among samples matrices and in Figure 5 at concentrations of 0.71 mg/mL and 0.87 mg/mL.

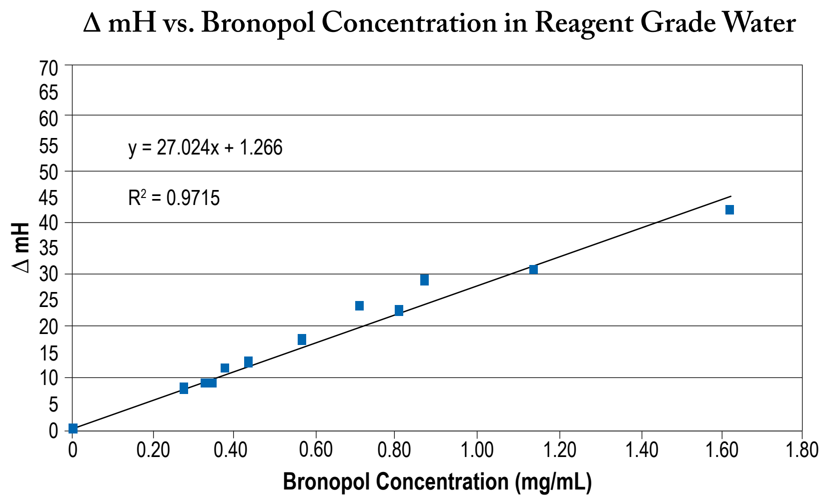

Fig. 2. Bronopol in reagent grade water

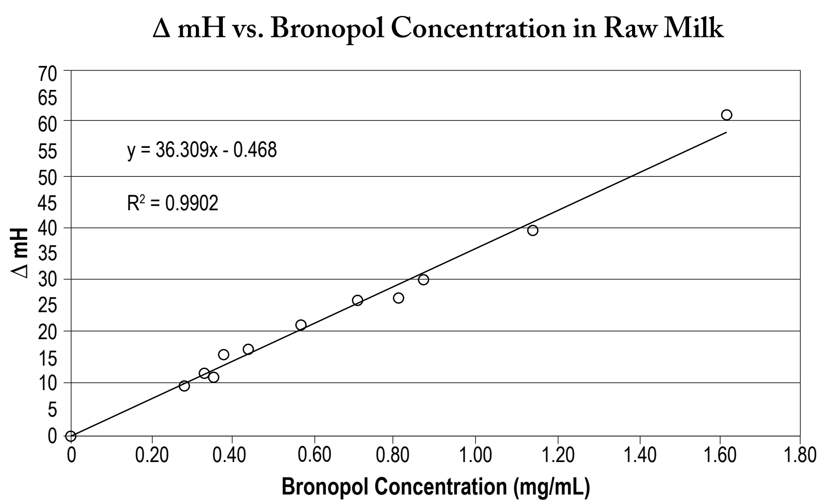

Fig. 3. Bronopol in raw milk

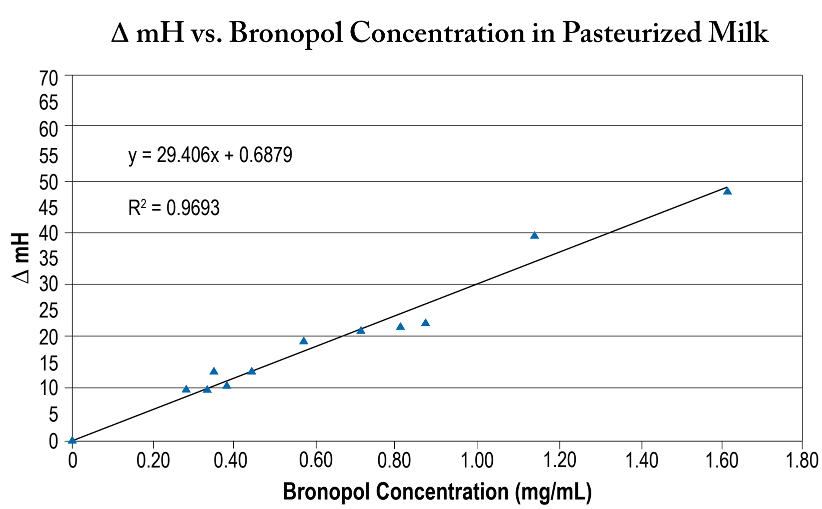

Fig. 4. Bronopol in pasteurized milk

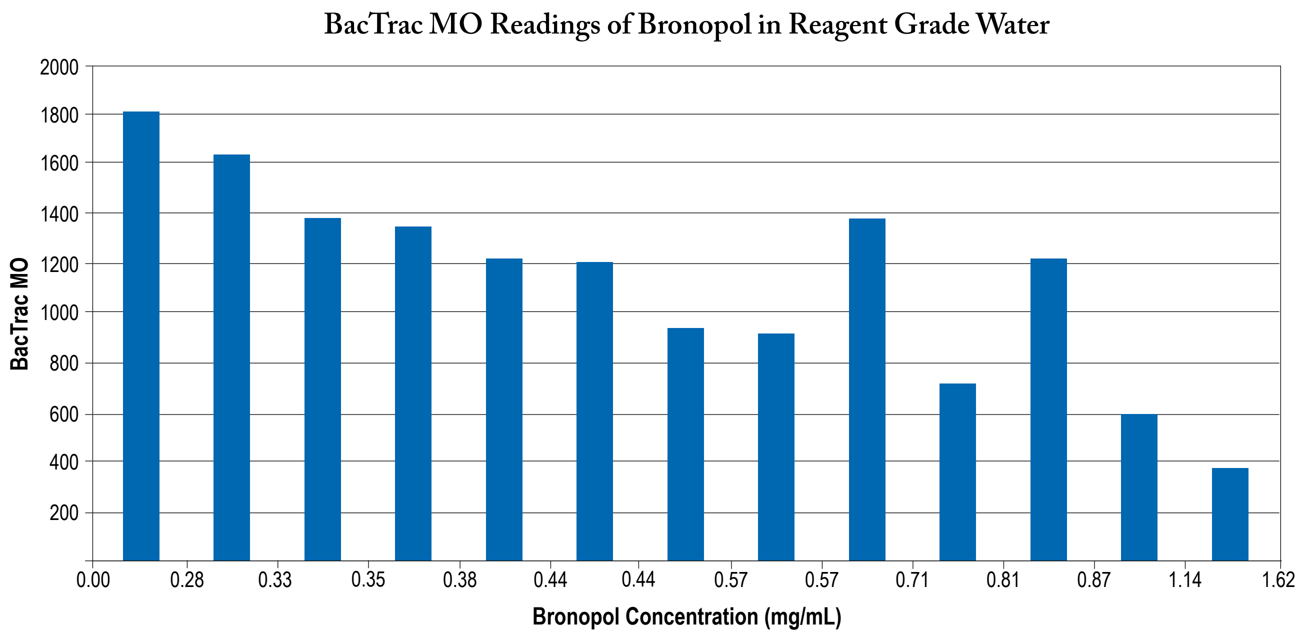

Fig. 5. Bronopol in reagent grade water

Sample #	Weight (mg)
1	15.6
2	19.2
3	15.7
4	17.8
5	16.0
6	17.6
7	14.7
8	16.4
9	16.0
10	18.9
Average	**16.8**
Std. Dev.	**1.5**
%CV	**8.95**

Table 2. Bronopol tablet weights

CONCLUSION

This study clearly demonstrates the quantitative effects of bronopol on freezing point and impedance measurements of milk. While it is highly recommended that users refrigerate and test unadulterated samples within 24 hours, preserved samples are an option when a suitable correction factor is applied.

ACKNOWLEDGEMENT

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