ADVANCED® APPLICATION NOTES

FOR THE FOOD AND DAIRY INDUSTRY JANUARY 2007

MONITORING LACTOSE HYDROLYSIS IN MILK AND MILK PRODUCTS WITH CRYOSCOPY

This method provides:

- 2-minute test time.
- Repeatability to ±0.5%.
- Elimination of filtrations, extractions or titrations.
- Elimination of additional reagents to mix or add.

The primary sugar found in milk is the disaccharide, lactose. In normal human digestion, lactose is broken down into two monosaccharides: glucose and galactose. Approximately 20% of the general population, however, is unable to adequately digest lactose. Until recently, lactose intolerant individuals have had to avoid dairy foods or suffer painful gastrointestinal symptoms. Today, lactose reduced milk and reduced calorie lactose free yogurt are commercially available. These products are often manufactured by the enzymatic hydrolysis of lactose, using the enzyme lactase.

Cryoscopy offers an easy and accurate analytical method for monitoring the commercial production of lactose reduced milk and other products. During enzmatic hydrolysis, lactose is broken down into galactose and glucose (Figure 1). The increase in the concentration of these two simple sugars causes a linear depression in the freezing point of the milk (1).

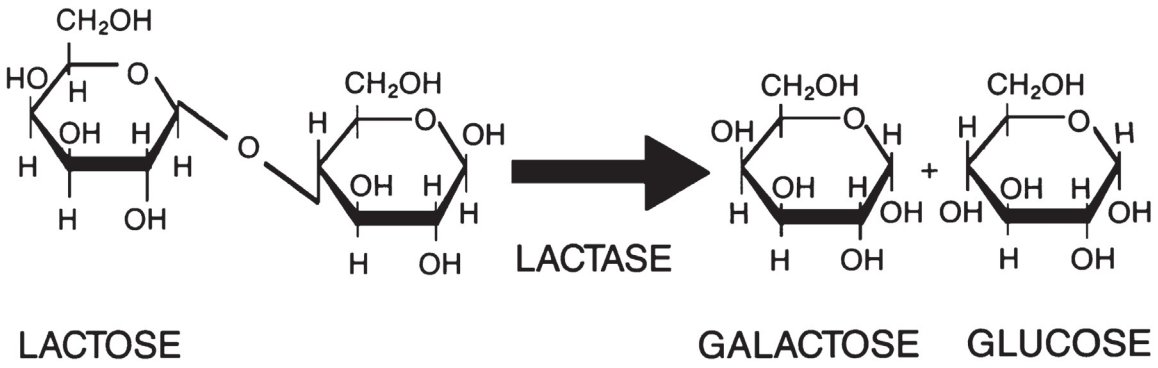

Figure 1. Enzymatic reduction of lactose in milk

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Chromatographic and spectrophometric techniques for measuring the progress of the enzymatic reduction of lactose require time-consuming sample preparation; the cryoscopic method eliminates these steps (1, 2).

The following procedure is adapted from a number of published methods which use cryoscopy to determine the amount of lactose hydrolysis that has occurred in an enzymatic reduction (3, 4).

Experimental Method

Reagents and Equipment:

1. Advanced Instruments Cryoscopes, Models 4250 and 4C3.
2. Lactase (*ß*-D-Galactoside) Galactohydrolase, EC3.2.1.23; from *Canadida pseudotropicalis*, from Pfizer, Inc., Milwaukee, WI 53212, or equivalent.
3. Aliquot of the milk being hydrolyzed (approximately 300 ml).

Procedure:

1. Calibrate the Cryoscope.
   Use Advanced Instruments Calibrators: 408 mºC (422 mºH) and 600 mºC (621 mºH). Calibrate the instrument in millidegree Celcius, according to the procedure specified in the Users Guide. Use the 408 mºC calibrator as the low standard, and the 600 mºC calibrator as the high standard. Check the linearity of the instrument with the 512 mºC Reference Solution.

   *In the United States, make sure your instrument is set to mºH.

2. Denature a small sample of the lactase.
   Pour a small sample of the lactase (approximately 10 ml) into a test tube. Place the test tube in a boiling water bath for approximately 10 minutes, or until the lactase is cloudy in appearance. Allow the lactase to cool to room temperature. This is the deactivated lactase required in the following steps.
3. Measure the freezing point depression.

A. *100% Hydrolyzed Sample Control*. Bring 1.0 ml of deactivated lactase to 100 ml with room temperature milk. Incubate at 37ºC for three hours. Measure the freezing point.

B. *100% Hydrolyzed Sample Test*. Bring 1.0 ml of lactase to 100 ml with room temperature milk and mix well. Incubate at 37ºC for three hours. Measure the freezing point.

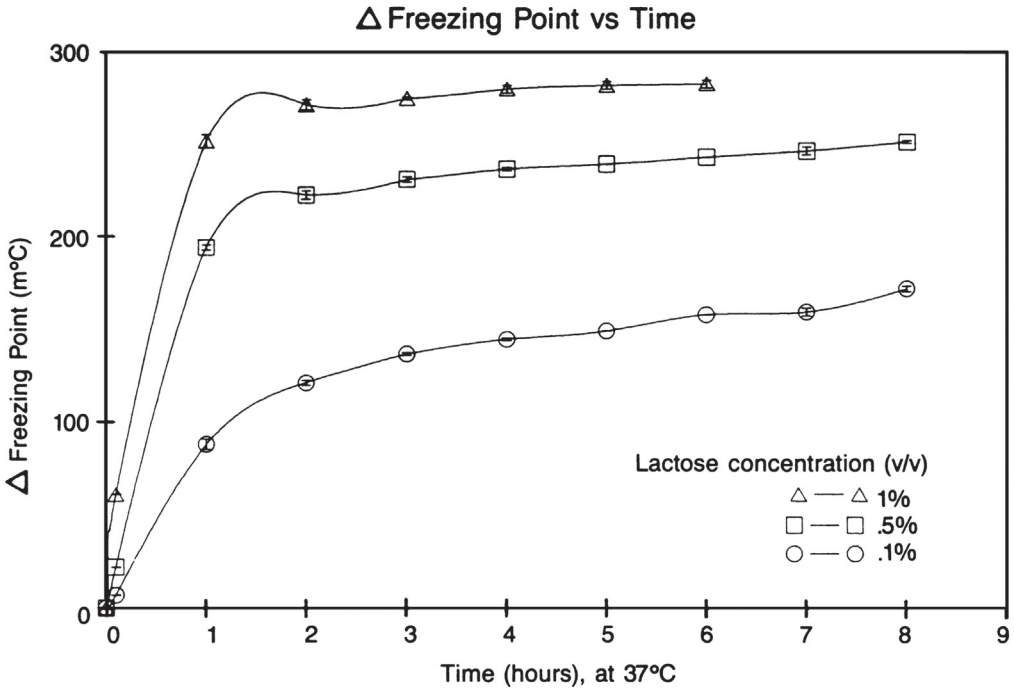

Figure 2. The effect of different concentrations of Pfizer Neutral Lactase on hydrolysis of lactose in regular whole milk

The decrease in freezing point caused by the conversion of lactose to glucose and galactose is determined by subtracting the freezing point of the 100% Hydrolyzed Sample Control (A) from the 100% Hydroloyzed Sample Test (B).

C. *% Hydrolyzed Production Sample*. Add lactase to product at the manufacturer's recommended concentration. [For example, for a 0.1% (v/v) solution, bring 0.1 ml lactase to 100 ml with product (at room temperature).] Incubate at 37ºC and measure the freezing point at regular intervals.

D. *% Hydrolyzed Production Sample Control*. Add deactivated lactase to the remainder of the aliquot of milk at the same concentration as the lactase in Step C. Incubate at 37ºC and measure the freezing point at regular intervals.

The decrease in freezing point that is a result of the conversion of lactose to glucose and galactose is determined by subtracting the freezing point for the % Hydrolyzed Production Sample Control (D) from the % Hydrolyzed Production Sample Test (C).

The percent of lactose that has been hydrolyzed at any interval can be calculated from the following simple equation.

$$\% \text{ Hydrolysis} = \frac{FP_C - FP_D}{FP_B - FP_A} (100\%)$$

Figure 3 shows a typical linear response that can be obtained using 0.1% Pfizer Neutral lactase, incubated at 37ºC and assayed for freezing point at regular intervals.

Conclusion: Cryoscopy is fast, economical and accurate.

This method provides the dairy processor with accurate results that serve as an immediate quality control check during production. No reagents are required by this procedure, and due to the direct nature of the test, there are no titrations, filtrations, or extractions to perform.

The Advanced Instruments cryoscopes provide an economical and easy means of evaluating lactose hydrolysis in milk.

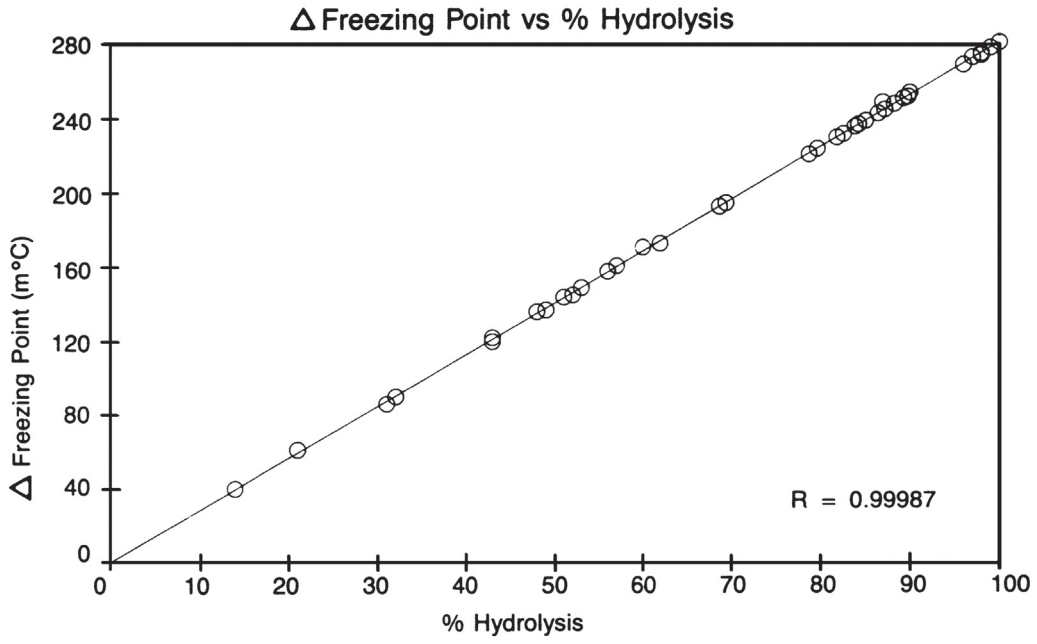

Figure 3. Whole milk was treated with 0.1% Pfizer Neutral Lactase, incubated at 37ºC and assayed for freezing point at regular intervals. Percent hydrolysis was calculated based upon a 100% hydrolyzed sample of whole milk (of the same lot).

References:

1. Kwak, H.S., and Jeon, I.J., *Comparison of High Performance Liquid Chromatography and Enzymatic Method for Measurement of Lactose in Milk*, J. Food Sci., 53:975-976 (1988).
2. Guy, Eugene J., and Bingham, E.W., *Properties of ß-Galactosidase of Saccharomyces lactis in Milk and Milk Products*, J. Dairy Sci., 61:147-151 (1978).
3. Baer, Robert J., et al., *Freezing Point Measurement of Lactose Hydrolysis in Acid Whey and Lactose Solutions*, J.O.A.C., 63:587-590 (1980).
4. Pfizer, Inc., *Cryoscopic Method for Determining Percent Hydrolysis*, Pfizer, Inc., Milwaukee, WI 53212 (12/11/84).

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