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Potential Implication of the Freezing Point Depression by Enzymatic Hydrolysis of Lactose in Milk[1]

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ABSTRACT

The potential problem of detecting added water in lactose-hydrolyzed milk by cryoscopic examination was investigated. The extent to which hydrolysis of lactose corresponded with a given freezing point was calculated and tested experimentally. Cryoscopic measurements were related to the percent of lactose hydrolyzed in milk. Hydrolyzed milks readjusted to normal freezing points with added water were examined by lactometer and sensory evaluations. Although such milk adulterated with up to 25% added water could escape detection by either cryoscopic or sensory evaluations, the Quevenne lactometer could detect 10% added water.

The dairy industry conventionally uses specific gravity measurements to screen milk for added water. However, confirmation of this adulteration by regulatory agencies where legal action may ensue is determined by freezing point depression. If a freezing point falls below -0.525 H (Hortvet), the milk is presumed to be free of added water (*1*). Although no lower limit has been established for the freezing point of milk, the accepted normal range is from -0.528 to -0.561 H (*2*). Recently, Nijpels et al. (*5*) reported that the degree of hydrolysis of lactose in milk is directly related to the depression of freezing point. When all the lactose in control milk was enzymatically hydrolyzed to monosaccharides, the freezing point was depressed -0.274 H.

Recent advances in the use of lactose hydrolysis for utilization of whey and production of low lactose milk have greatly enhanced the economic availability of lactase. Regulatory agencies should be alerted to the effect hydrolysis of lactose has on the freezing point of milk, particularly on milk adulterated with water. The objective of this report is to share our observations on the effect that adding water to lactose-hydrolyzed milk has on freezing point, lactometer reading, and sensory evaluation.

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MATERIALS AND METHODS

Lactose-hydrolyzed milk was prepared by adding 1.0 ml of lactase solution (Pfizer's experimental lactase extracted from yeast) to 1 l of pasteurized milk. This was stored at 6 C for approximately 24 h. The lactose-hydrolyzed milk then was mixed with control (untreated) milk to achieve a series of the desired amounts of hydrolysis. The degree of hydrolysis in the mixture was determined by measuring the depression of its freezing point (*5*) with Fiske milk cryoscope Model J-61 (Fiske Associates, Inc., Uxbridge, MA). To confirm the level of hydrolysis in the mixture, a modified o-toluidine method was used (*3*). In this chemical method, all milk samples and standard sugar solutions (glucose, galactose and lactose) were analyzed simultaneously in duplicate with a Beckman DU-2 spectrophotometer. Average absorbance values of the three sugars were used to calculate sugar concentrations in milk for the % hydrolysis of lactose. To support further use of the freezing point method to measure the extent of hydrolysis in the mixture of the lactose hydrolyzed and control milk, a series of aqueous standard solutions containing glucose, galactose, and lactose was prepared at the molar concentrations with each sugar equivalent to a 5% lactose solution hydrolyzed at 0, 25, 50, 75 and 100%. Freezing points of each of the standard solutions with the aforementioned concentrations of sugars were measured.

In another set of experiments, lactose-hydrolyzed milk was mixed with control (untreated) milk, as before, to obtain a desired level of hydrolysis (Table 1). In this case, however, the degree of hydrolysis was determined only by measuring the depression of freezing point. A certain amount of water was then added to the mixture to adjust the freezing point back to near its original level (before hydrolysis). After adding water, we again measured the freezing point as well as determined the Quevenne lactometer value at 15.6 C for each mixture of sample. Additionally an eight-member, untrained taste panel using a triangle taste test (*4*) attempted to distinguish the water-diluted milks from normal milks.

RESULTS AND DISCUSSION

Figure 1 shows the regression line that relates % hydrolysis in milk as determined by chemical analysis to the depression of freezing point. Superimposed upon that line are freezing points of standard sugar solutions representing sugar concentrations equivalent to 0, 25, 50, 75 and 100% lactose hydrolysis. Furthermore, the regression line agrees well with the theoretical freezing point difference of -0.273 H between the 0 and 100%

TABLE 1. *Effect of adding water to lactose-hydrolyzed milk on freezing point and lactometer readings.*

Lactose-hydrolyzed milk			Water added to lactose-hydrolyzed milk			
Degree hydrolysis (%)	Freezing point[1] Calculated[2]	Freezing point[1] Achieved[3]	Water added to milk By ratio	Water added to milk By percent	Resultant freezing point[1]	Quevenne reading
0	-0.543	-0.543	0	0	-0.543	32.5
22	-0.603	-0.604	0.110	9.9	-0.540	29.2
35	-0.639	-0.641	0.175	14.9	-0.538	27.5
50	-0.680	-0.685	0.250	20.0	-0.537	26.3
67	-0.726	-0.726	0.335	25.1	-0.536	24.5
85	-0.775	-0.775	0.425	29.8	-0.535	22.3

[1]H (Hortvet) unit.
[2]Calculated from the freezing point depression curve (Figure 1).
[3]Achieved by mixing the lactose-hydrolyzed milk with control (untreated) milk.

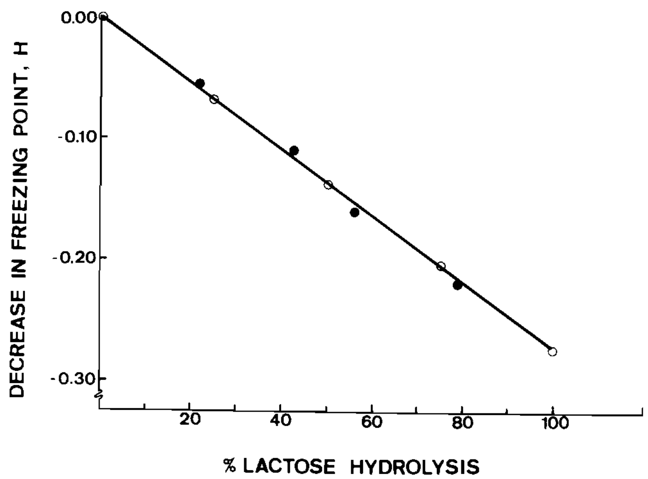

Figure 1. *Linear relationship between the depression of freezing point and percent of hydrolysis of lactose. ●- Percent of lactose hydrolysis determined by chemical analysis of milk. O- Represents standard sugar solutions containing glucose, galactose, and lactose prepared at molar concentrations equivalent to a solution of 5% lactose hydrolyzed at 0, 25, 50, 75 and 100%. H = Hortvet unit.*

hydrolysis of lactose (50 g/liter base) calculated by Nijpels et al. (5). For practical purposes, milk comparable to any desired level of lactose hydrolysis can be prepared, according to the linear relationship between the depression of freezing points and the % hydrolysis, by mixing milks with low and high freezing points. Based on this relationship, freezing points for lactose-hydrolyzed milk were calculated with respect to the % of lactose hydrolyzed and were found to compare favorably with the freezing points actually achieved by mixing lactose-hydrolyzed and control milks (Table 1).

As shown in Table 1, by careful manipulation one can add a considerable amount of water to lactose-hydrolyzed milk and still maintain freezing points within the normal range for milk. For example, the freezing point of milk with 30% added water (85% hydrolysis) is -0.535H which is well within the normal range (-0.528 to -0.561 H) for milk. The water-to-milk ratio needed to raise the freezing point back to near the original point (before hydrolysis) is about half of the % hydrolysis divided by 100. For example, a 22% hydrolyzed milk would require 11 parts of water to 100 parts of milk to adjust it to near its original freezing point. The lactometer readings could detect such adulterated milk at about 10% added water. It is noteworthy to mention that the added water could be detected by sophisticated modern instruments (e.g., Infrared milk analyzer) capable of measuring individual components in milk.

Surprisingly, our taste panel, using the triangle test, failed to distinguish between control milk (undiluted) and the hydrolyzed milk diluted with water to near its original freezing point. Only 4 of 8 panelists correctly identified milk with 20% added water (50% hydrolysis) and only 5 persons detected 25% added water (67% hydrolysis). For an eight-member triangle test, the results of correct answers are not significant ($p>0.05$). It seems that, although hydrolyzed milk is sweeter, its sweetness is reduced with added water; however, the milk is still sweet enough to mask the flat taste associated with added water. Apparently neither cryoscopic measurements nor sensory tasting can easily detect added water in lactose-hydrolyzed milk. It is important that regulatory agencies recognize that they may need other means to detect adulteration of milk with water (e.g., specific gravity) as the use of lactase becomes more prevalent.

REFERENCES

1. Association of Official Analytical Chemists. 1980. Official methods of analysis, 13th ed. Association of Official Analytical Chemists. Washington, D.C.
2. Atherton, H. V., and J. A. Newlander. 1977. Chemistry and testing of dairy products. AVI Publishing Co., Westport, Conn.
3. Dubowski, K. M. 1962. An o-toluidine method for body fluid glucose determination. Clin. Chem. 8:215-235.
4. Larmond, E. 1977. Laboratory methods for sensory evaluation of food. Canada Department of Agriculture, Publication 1637. Ottawa, Ont., Canada.
5. Nijpels, H. H., P. H. Evers, G. Novak, and J. P. Ramet. 1980. Application of cryoscopy for the measurement of enzymatic hydrolysis of lactose. J. Food Sci. 45:1684-1687.