# CHAPTER III

# LACTOSE HYDROLYSIS BY YEAST LACTASE: INFLUENCE ON FREEZING POINT AND DIPPING CHARACTERISTICS OF ICE CREAM

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## ABSTRACT

Ice cream mixes were treated with lactase (EC 3.2.1.23) to cause 0 to 83% lactose hydrolysis. Lactose hydrolysis decreased the freezing point from -1.63°C in the control (0% hydrolyzed) samples to -1.74°C in the 83% hydrolyzed sample ($p < 0.05$). Firmness decreased from 0.35 J in the control sample to 0.08 J in the 83% hydrolyzed sample. Lactose hydrolyzed samples melted at a faster rate than the control. There was a difference ($p < 0.05$) in ease of dipping between samples treated with lactase and the control. There were no perceived differences in sweetness and coldness.

**Key Words:** dippability, freezing point depression, ice cream, lactose hydrolysis

# INTRODUCTION

The potential market for lactose-hydrolyzed foods has been recognized by the industry since the 1950's (Holsinger and Kligerman, 1991) but the process did not become economically feasible until the commercial development of the enzyme β-galactosidase from microbial sources (yeast, bacterial, and/or fungal) in the seventies (Anon., 1984). Enzymes from microbial sources are of technological interest because they produce high yields of enzyme (Cavaille and Combes, 1995). For commercial operations, microbial sources which enable large amounts of the suitable enzyme to be conveniently extracted are the most desirable (Palmer, 1985). The cost of enzymes are related to purity; however, the relative cost of the entire hydrolysis process is continually changing with advances in technology (Zadow, 1986). The process increases the cost of product manufacturing so the market potential must be analyzed to determine profitability of certain products (Marshall, 1991).

Lactose makes up over one third of the solid matter in milk, and approximately 20% of the carbohydrates in ice cream (Marshall and Arbuckle, 1996). The percentage of lactose in ice cream is dependent on the amount of milk solids not fat (MSNF) and fat in the mixture. The level of MSNF in ice cream mix is limited by the potential contribution of lactose crystallization which causes sandiness in frozen desserts. Superpremium types of ice cream have a higher percentage of fat and the source of MSNF are usually limited to skim milk solids. Whey solids are made up of about 72% lactose; skim milk solids have about 50% less lactose than whey solids (Marshall and Arbuckle, 1996). Whey solids are used to replace skim milk solids in economy ice cream. Therefore, economy-type ice creams have more lactose (Marshall and Arbuckle, 1996).

Lactose is hydrolyzed by enzymes which are used to break down the disaccharide into its constituent monosaccharides; D-glucose and D-galactose. This results in an increased amount of soluble solutes in solution which lowers the freezing point of the solution (Iversen, 1983). Substituting monosaccharides for higher molecular weight sugars will also increase the amount of solids in the mix and depress the freezing point. Freezing point in ice cream is influenced by the major components of low molecular weight; i.e. milk salts, sugar, corn syrup solids, and milk sugar (Mitchell, 1989). It is directly proportional to the number of particles in solution (Iversen, 1983; Mitchell, 1989). The more solids dissolved in the solution, the lower the freezing point. The composition and concentration of the soluble constituents change with different mix formulations, therefore affecting the freezing point (Marshall and Arbuckle, 1996).

Ice cream hardness and melting characteristics are important for improved dipping characteristics. Factors that can enhance the softness and extrudability of ice cream are: (1) increasing the amount of air whipped into the product (overrun), (2) depressing the freezing point, and (3) temporarily increasing the storage temperature (Iversen, 1983; Lindamood et. al, 1989). Lindamood et al. (1989) conducted a study to determine the effects of lactose and sucrose hydrolysis on freezing point of ice cream. The changes in texture, firmness, freezing point, melting characteristics and relative sweetness were assessed. Results relative to lactose hydrolysis were confounded because sucrose hydrolysis occurred simultaneously. A commercially available lactase (Lactaid brand, McNeil Specialties, Inc. Pleasantville, N.J.) was used for lactose hydrolysis. Sucrose hydrolysis occurred at a faster rate than lactose hydrolysis. The ice cream mix

treated with lactase to achieve 25% lactose hydrolysis was determined to have 22.4% lactose hydrolysis and 15.1% sucrose hydrolysis. The freezing point was calculated to be -1.62$^{o}$C. The mixes treated with lactase to achieve 50% and 100% lactose hydrolysis had 44.0% and 78.4% lactose hydrolysis, respectively, and 7.6% and 22.5% sucrose hydrolysis, respectively. Calculated freezing points for these mixes were -1.67$^{o}$C and -1.92$^{o}$C, respectively. It was concluded that the lactase enzyme preparation was not lactose specific. Enzyme purification is important to ensure that no unwanted reactions occur (Palmer, 1985).

Limited work has been reported on the influence of lactose hydrolysis on physical and sensory characteristics of ice cream. Physical measurements of firmness have been used to estimate the ease of dipping ice cream (Lindamood et al., 1989). Lindamood et al. (1989) evaluated the firmness of 0%, 25%, 50% and 100% lactase treated ice cream samples (~ -15$^{o}$C) by compression testing using a universal testing machine (Instron, Model 100, Canton, MA). The measure of relative firmness was determined by a curve generated as force versus distance and work was calculated as the area under the curve. Non-hydrolyzed ice cream samples had a relative firmness of 0.44 J. The samples treated with lactase (25%, 50% and 100%, respectively) were shown to be different ($p < 0.05$) from that of the control sample (0.29 J, 0.18 J, 0.13 J, respectively). It was determined that untreated samples were more firm than samples treated with the lactase enzyme.

Relative sweetness of lactose is approximately 20% that of sucrose whereas glucose and of galactose are 60% as sweet as sucrose (Fennema, 1996; Iversen, 1983). According to Zadow (1986), hydrolysis of 70% of the lactose in milk increases its sweetness by an amount comparable to the addition of approximately 2% sucrose. Lindamood et al. (1989) reported that neither an untrained taste panel nor trained judges criticized that the lactose-hydrolyzed ice cream samples were excessively sweet. Calculated relative sweetness for the non-hydrolyzed samples was 16.0 and the lactase treated samples (25%, 50%, and 75%) were 16.7, 17.2, and 18.5, respectively. This present study utilizes pure lactase enzymes to hydrolyze the lactose in an ice cream mix. The effects of lactose hydrolysis on freezing point and texture were measured. Human perception of ease of dipping and changes in perceived sweetness and coldness were also evaluated.

## MATERIALS AND METHODS

### Ice Cream Mix

Extended shelf-life ice cream mix (Shenandoah's Pride Dairy, Mt. Crawford, VA) was used for this study. The formulation of the mix was 10% fat, 11% MSNF, 15% sucrose, and 0.3% stabilizer. Mix was stored at 4°C until it was frozen.

### Lactose Hydrolysis of Ice Cream Mix

Lactose hydrolysis was accomplished by an enzyme preparation from *Kluyveromyces lactis* (Validase Yeast Lactase Lot #CQ7231, Valley Research, South Bend, IN). *Kluyveromyces lactis* is a yeast that produces one of the most widely used lactose hydrolyzing enzymes (Holsinger and Kligerman, 1991). The pH optimum of this enzyme is 6.9-7.2; the pH at which the enzyme is stable is 7.0-7.5; and the temperature optimum is 35°C (Greenberg and Mahoney, 1981). Enzyme was stored at 4°C until addition to the ice cream mix. Enzyme preparation was filtered through sterile 0.45 μm acrodiscs (Gelman Sciences prod. no 4184, Fisher Scientific, Pittsburgh, PA) and added to 9.1 L (2 gal) ice cream mix. Quantity of enzyme added was dependent on the desired degree of hydrolysis to be achieved (0% (control), 25%, 50% and 75%). Lactase activity is determined by blending a diluted enzyme sample with a 0.005 M preparation of o-nitrophenyl-β-D-galactopyranoside (ONPG)(Shah and Jelen, 1990). The amount of o-nitrophenyl released is measured and the lactase activity is estimated as the amount of enzyme which liberated one μmole o-nitrophenyl from ONPG per minute per gram samples at 37°C (Shah and Jelen, 1990). The activity level of the enzyme was 53,000 ONPG.

The lactase enzyme from the fungal source *Aspergillus oryzae* was also used in preliminary tests also. It was determined that not only did it require approximately four times the amount of enzyme to achieve the same amount of hydrolysis as the yeast-derived enzyme, but the enzyme imparted an off-flavor to the ice cream. The pH of an ice cream mix with 11% MSNF is 6.31 (Marshall and Arbuckle, 1996). Lactase from *Aspergillus oryzae* is an "acid" lactase, which have a pH optimum around 4.5 and a temperature optimum of 55°C, whereas "neutral" lactases like *Kluyveromyces lactis* show an activity optimum between pH 6.0 and 6.5 and temperatures between 36°C and 38°C (Jelen, 1993). The cold storage temperature and neutral pH of the ice cream mix were probably responsible for the low activity level of the fungal-based enzyme.

Preliminary testing determined that 0.61 mL, 1.5 mL, and 6.1 mL, of enzyme respectively, achieved the targeted 25%, 50%, and 75% hydrolysis of lactose in 9.1 L of mix in 24 h at 4°C. These levels were determined by inoculating 1 L of mix with various levels of enzyme and then testing the amount of lactose hydrolysis achieved after 24 h of storage at 4°C. Enzyme-inoculated ice cream mixes were held for 24 h at 4°C and agitated with a stirring rod for one min at two different intervals during the storage period to disperse the enzyme. The degree of hydrolysis achieved after 24 h was determined based on the amount of lactose and D-galactose in the sample (Lactose/D-Galactose test kit, Boehringer Mannheim, Indianapolis) as measured spectrophotometrically (Spectronic 1001 Split Beam Spectrophotometer, Milton Roy Company, Rochester, NY). Sucrose concentration was also assessed (Sucrose/D-Glucose test kit, Boehringer Mannheim, Indianapolis) to determine if any hydrolysis occurred as an indication of the specificity of

the enzyme preparation. Sample preparation was described by Wu et al. (1996) and the Boehringer Mannheim test kit information (Sucrose/D-Glucose test kit, Boehringer Mannheim, Indianapolis) for the lactose and sucrose assays, respectively.

**Freezing the Ice Cream Mix**

Ice cream mixes (0%, 25%, 50%, 75% hydrolysis) were frozen (Emory Thompson Freezer 2HSC A, Emory Thompson Machine and Supply Co., New York) with approximately 75% overrun. Time required for freezing and the percentage of overrun were standardized as much as possible. For each treatment the freezing point was determined using a thermistor cryoscope (Advanced Milk Cryoscope Model 4C, Advanced Instrument Inc., MA) and recorded in degree Hortvet. Samples were diluted with three parts of water to one part of mix (Ohmes et al., 1998). Degrees Hortvet ($^oH$) were converted to degrees Celsius using the following equation: $^oC = 0.9\ (^oH - 0.0024)$(dilution factor) (Ohmes et al., 1998). Percentage overrun was determined as described by Marshall and Arbuckle (1996). Ice cream was packaged in 0.18 L (6 oz) plastic freezer containers (Sweetheart Plastic Food Cups, Sweetheart Cup Company, Inc., Chicago) for texture tests. A 4.6 L (1 gal) container (Tucker Housewares, Division of Zeta Consumer Products Corp., Leominster, MA) of ice cream was used for the sensory tests. All containers were fitted with lids and immediately transferred to a freezer ($-20.0^oC$) for hardening and storage.

**Evaluation of Mix and Frozen Ice Cream**

Gross composition of the mix (total fat, protein, moisture, total solids) were evaluated to verify consistency among replications. Fat content was determined using the Pennsylvania modified Babcock method (Marshall, 1993). Protein content was determined by the Bradford method (Bio-Rad protein assay, Bio-Rad, Hercules, CA) using a spectrophotometer (Spectronic 1001 Split Beam Spectrophotometer, Milton Roy Company, Rochester, NY). Moisture content and total solids were determined using an infrared analyzer (Infrared Analyzer 115 Vac, Denver Instrument Company, Arvado, CO).

Microbial load of the hydrolyzed mix was determined for each repetition after the addition of the enzyme and before freezing the mix. This was accomplished by diluting the samples (1:10 and 1:100) in a dairy dilution blank made up of phosphate and magnesium chloride in distilled water (Marshall, 1993). An undiluted sample (1 mL) and each diluted sample (1 mL) was plated on aerobic count Petrifilm (3M Petrifilm, Microbiology Products 3M Health Care, St. Paul MN) and coliform count Petrifilm (3M Petrifilm, Microbiology Products 3M Health Care, St. Paul MN). Standard plate counts were incubated at $32^oC$ for 48 h, and psychrotrophic bacteria counts were incubated at $7\ ^oC$ for 10 days on aerobic count Petrifilm (Marshall, 1993). Coliform counts were incubated at $32\ ^oC$ for 24 h on coliform count Petrifilm (Marshall, 1993).

Melting and sensory characteristics of the ice cream treatments were determined within two weeks of storage at $-20.0^oC$. Melting characteristics were evaluated for melting defects and assessed using the meltdown test described in Arbuckle (1986). A uniform block of frozen ($\sim -16.5^oC$) ice cream (0.18 L volume) was placed on a #10 wire sieve (25.4 wires/cm) maintained at $20^oC$. The time required for 10 g to melt off the

block was noted. For every 5 minute period following this initial collection, the collected liquid was weighed and compared against time. This was recorded as meltdown g/min.

Ranking tests (Meilgaard et al., 1991) were used to determine the sensory characteristics of sweetness and coldness. For this test, 24 untrained panelists, recruited from the faculty, staff and students of the Food Science and Technology Department, were used to evaluate each treatment of ice cream with 0%, 25%, 50% and 75% lactose hydrolysis. The samples were placed in 28 g (1 oz) plastic cups (Solo Cup Company, Urbana, IL), fitted with lids, and assigned randomly selected 3 digit codes. The samples were presented in a balanced order under white lighting. Panelists were seated in isolated sensory booths. Panelists ranked the samples in order of increasing sweetness and increasing coldness (1 = least sweet, 4 = most sweet; 1 = least cold, 4 = most cold). Data from three replications were pooled and analyzed using the Friedman's test (5% level of significance)(Meilgaard et al., 1991).

Textural characteristics were determined analytically by instrumental methods and human perceptual methods. Firmness of the frozen ice cream was determined analytically following the method described by Lindamood et al. (1989). Samples from all three replications were evaluated on the same day to ensure similar test conditions. These analyses were conducted using an Instron (Model 100, Instron, Canton, MA). A cylindrical probe (6.4 mm), pre-cooled in ice water, was mounted on a 5 kg transducer to take compression measurements of ice cream samples (~ -16.5$^{o}$C). Cross-head speed was set at 20 mm/min for a depression of 10 mm. Firmness was measured as energy, and converted to Joules using the equation: energy (gm-cm) x 9.80665 x $10^{-5}$ = Joules (Weast and Selby, 1967).

Texture was also measured using human subjects to determine the ease of dipping for each hydrolysis treatment. Sixteen panelists were recruited from the Food Science and Technology Department and shown the proper way to dip ice cream (Marshall and Arbuckle, 1996). Reference samples of "extremely easy to dip" and "extremely difficult to dip" ice creams (Breyer's Soft and Creamy Vanilla (Good Humor-Breyer's, Green Bay, WI) and World's Best Vanilla (Ben & Jerry's Homemade Inc., Burlington, VT), respectively) were provided as reference points. Testing was conducted in the Dairy Processing Plant (Department of Food Science and Technology, Virginia Tech).

Panelists dipped ice cream samples contained in 4.6 L containers from a cooler maintained at -16.5$^{o}$C. The samples were assigned a three digit code and panelists were instructed to dip samples in an assigned randomized order. The panelists dipped from each treatment and ranked the samples in order of their ease of dippability (1 = easiest to dip and 4 = hardest to dip). The data from this test was analyzed using the Friedman's test (5% level of significance).

**Statistical Analysis**

The complete experiment was replicated three times. For each enzyme treatment, four levels of hydrolysis were analyzed. At least two observations (duplicate measurement) for each analysis (fat, protein, lactose, moisture, total solids, firmness, rate of melting, freezing point) were taken.

Linear regression was completed for each dependent variable using Microsoft Excel ‘97 software (Microsoft Corporation, Redmond, WA). The Statistical Analysis System (SAS Institute Inc., 1988) software was used to conduct analysis of variance on

firmness, rate of melting, and freezing point data. Significance was decided at a predetermined alpha of 0.05. Tukey's test was used to determine mean separations when significance ($p < 0.05$) was observed.

For each of the three replications, sixteen independent observations (number of panelists) were utilized for dippability testing and 24 observations were utilized for the "Sweetness" and "Coldness" sensory tests. The observations from three replications were pooled together and Friedman's analysis was used to analyze the results. Results from the sensory dippability study were compared to the firmness data.

## RESULTS AND DISCUSSION

### Composition of Ice Cream

The mean fat content of the ice cream mix was verified as 10%. The average protein content of the mix was 15.76 +/- 1.31 mg/mL mix. Percentage moisture and total solids were 66.05 +/- 0.583% and 33.95 +/- 0.583%, respectively. The results from the microbial counts were below detectable limits. During freezing, overrun was maintained at 73 +/- 0.81% for all replications.

### Hydrolysis

Although specific levels of hydrolysis were targeted, variations in the degree of lactose hydrolysis existed (Table 1). Hydrolysis levels ranged from 28 +/- 4.4% for the target hydrolysis level of 25%, 43 +/- 8.8% for the target level of 50%, and 71 +/- 6.4% for the target level of 75%. The inconsistency of lactose hydrolysis may be due to uneven dispersion of the enzyme in the mix. The viscosity of the mix may have limited the proximity of the enzyme to the substrate during intervals subsequent to mixing. During preliminary trials, low levels of enzyme were added to 1 L containers of mix and shaken by hand for 30 sec. When the lot size was increased to 9.1 L it may not have been possible to disperse the enzyme as completely, resulting in variable hydrolysis levels. Later studies by Matak (1999) indicated that hydrolyzing 100% of the lactose in small volumes of ice cream mix and diluting it by adding unhydrolyzed mix was a more accurate method of achieving target hydrolysis levels than the addition of a specific dose of enzyme. Sucrose hydrolysis was assessed to determine the specificity of the lactase enzyme (Table 1). There was no significant hydrolysis of sucrose and the enzyme was considered to be lactose specific.

Freezing points averaged -1.63 +/- 0.01$^{o}$C in the untreated samples to -1.72 +/- 0.01 for samples with high levels of lactose hydrolysis (Table 2). A negative relationship existed between degree of lactose hydrolysis and freezing point depression ($R^2 = 0.61$; $p < 0.05$)(Fig. 1). Marshall and Arbuckle (1996) reported that typical ice cream mix containing 12% fat, 11% milk solids not fat (MSNF), 15% sugar, 0.3% stabilizer, and 61.7% water has a freezing point of approximately -2.5$^{o}$C. Lindamood et al. (1989) calculated freezing point by the methods of Iversen (1983). It was calculated that an ice cream containing 10% milkfat, 12% milk solids-not fat, 12% sucrose, 5% corn syrup solids (36 DE) and 0.25% stabilizer-emulsifier blend would have a freezing point of -1.45$^{o}$C. Mixes hydrolyzed to 25%, 50%, and 100% had calculated freezing points of -1.62$^{o}$C, -1.67$^{o}$C, and -1.92$^{o}$C, respectively. Sucrose hydrolysis in their study may have affected changes in freezing point. Differences may have also been due to the variations in sugar compositions of the mixes used in each study.

Melting rate is considered an important quality to consumers (Marshall and Arbuckle, 1996). An ice cream that melts too fast is undesirable because it becomes heat shocked readily (Marshall and Arbuckle, 1996). When environmental conditions are equal the primary cause of this defect is low freezing point (Marshall and Arbuckle, 1996). Ice cream that melts too slowly is also considered a defect. Ice cream that displays desirable melting qualities will show unmistakable signs of melting 10-15 min after having been dipped and placed at room temperature (Marshall and Arbuckle, 1996). Results of the meltdown test demonstrate that the control treatment (0% hydrolysis) took

the longest amount of time to melt (1.43 +/- 0.05 mL/min) and the 75% hydrolyzed ice cream took the shortest amount of time (1.67 +/- 0.01 mL/min)(Table 2). The 25% and 50% lactose hydrolyzed samples melted at a rate of 1.50 +/- 0.07 and 1.56 +/- 0.01, respectively. These rates were much faster than the ones reported by Lindamood et al. (1989). Lindamood et al. (1989) observed that the rate of melting an ice cream with 0% hydrolysis was 0.45 mL/min. Melting rates of 25%, 50% and 100% lactose hydrolyzed ice creams were reported as 0.32 mL/min, 0.82 mL/min, and 0.81 mL/min, respectively. The differences in melting rates may have been caused by differences in initial sample sizes. The core of the smaller sample size as used in this study may have reached a higher temperature faster than then that of the larger sample resulting in faster melting. The difference in melting rates may have also been attributed to differences in the formulations of the mixes. Results of the meltdown test imply a positive relationship between the degree of lactose hydrolysis and melting rate ($R^2 = 0.80$; $p < 0.05$)(Fig. 2). Even though there was a difference in melting rates of the hydrolyzed ice creams, there were no observed melting defects such as curdy, does not melt, flaky, foamy, wheying off or low viscosity (Marshall and Arbuckle, 1996).

**Sensory Evaluation**

Lactose reduction in ice cream samples did not alter perception of sweetness ($p > 0.05$). Sutton et al. (1995) demonstrated that there was no increase in perceived sweetness among custard samples that were treated with lactase. Sutton et al. (1995) theorized that in a product whose sweetness is derived in part from sucrose and is affected by other ingredients, such as vanilla, the increase of glucose and galactose resulting from lactose hydrolysis is not enough to make an obvious change in sweetness. Ice cream samples with levels of lactose and/or sucrose hydrolysis that ranged from 0% to 78% were evaluated by an untrained panel (Lindamood et al., 1989). The samples were not judged to be too sweet.

Differences in coldness of ice cream samples with varying freezing points were not perceived by the sensory panel ($p > 0.05$). Freezing point changes due to lactose hydrolysis were different by hundredths of degrees. The samples were presented at the same temperature and the variations in freezing points were too slight to be noticed by human senses.

**Textural Studies**

The differences in firmness between hardened ice creams from mixes hydrolyzed to different degrees are illustrated in Table 2. Firmness was measured by work (joules) required to penetrate with a pre-cooled cylindrical probe an ice cream sample held between -16.5$^{o}$C and -18$^{o}$C. As the degree of hydrolysis increased, the firmness of the ice cream decreased ($p < 0.05$). There was a significant difference between the firmness of the control sample and each of the treatment groups. It was also determined that there was a significant difference in firmness between the 25% and 75% hydrolyzed samples. Firmness of ice cream was inversely related to the degree of lactose hydrolysis ($R^2 = 0.70$; Figure 3). Although a low $R^2$ value was obtained, it was believed that temperature variations were the cause. Matak (1999) reported that firmness measurements were temperature dependent ($R^2 = 0.98$). A temperature difference as small as a tenth of a degree in samples could affect the firmness measurement. Lindamood et al. (1989) found

that non-hydrolyzed ice cream samples had a relative firmness of 0.44 J. The samples treated with lactase (25%, 50% and 100%, respectively) were different ($p < 0.05$) from that of the control sample (0.29 J, 0.18 J, 0.13 J, respectively). The results reported from Lindamood et al. (1989) were firmer then that of the present study. These differences may be due to variations in mix formulations and freezing points.

Firmness measurements of two commercial brands of ice cream were taken for comparison. An "extremely difficult to dip" and an "extremely easy to dip" vanilla ice cream ((World's Best Vanilla, Ben & Jerry's Homemade Inc., Burlington, VT) and (Breyer's Soft and Creamy Vanilla, Good Humor-Breyer's, Green Bay, WI)) were tested under the conditions as described above. Firmness measurements for the "extremely difficult to dip" and the "extremely easy to dip" averaged 0.51 J and 0.04 J, respectively. The "extremely difficult to dip" ice cream was expected to be firmer because of the high fat content and low overrun. The "extremely easy to dip" was expected to be softer because of increased amounts of monosaccharides in the mix and high percentage of overrun.

Lindamood et al. (1989) suggested that ice cream softness and extrudability could be enhanced by freezing point depression. In our study, there was a statistically significant difference ($p < 0.05$) for ease of dipping between the control (0% hydrolysis) and each sample treated with the lactase enzyme (Table 2). Samples treated with the higher amounts of lactase were consistently ranked “easier to dip” than the control sample which was not treated with the enzyme. There was also a difference between the 25% hydrolyzed group and the 75% hydrolyzed group. On a four point ranking scale (1 = easiest to dip and 4 = hardest to dip), the control had an average rank of 3.33, and the samples targeted with 25%, 50%, and 75% hydrolysis had an average rank of 2.56, 2.31, and 1.79, respectively.

## CONCLUSION

The ice cream industry today is using the incorporation of additional monosaccharides into ice cream mix to increase the amount of soluble solutes in the solution. This is done to depress the freezing point to result in a softer, more extrudable product. The hydrolysis of the milk sugar lactose is another way to increase the amount of soluble solutes in solution without contributing additional calories to the product. The observed effect of lactose hydrolysis on the firmness of ice cream is significant. The hydrolysis of lactose results in a narrow range of freezing point depression and the monitoring of this attribute is not enough to determine changes in dipping characteristics. The implication of this research on industry allows for the use of lactase enzymes to create an ice cream with enhanced dipping characteristics at low temperatures without excessive changes in melting character or sweetness.

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**Table 1.** Hydrolysis levels of ice cream mixes with various levels of lactase enzyme.

| Target lactose hydrolysis[a] (%) | Amount of enzyme added to 9.1 L ice cream mix (mL) | Percent lactose hydrolysis (mean[b] +/- s.e.) | Percent sucrose hydrolysis (mean[b] +/- s.e.) |
|---|---|---|---|
| 25 | 0.6 | 28 +/- 4.4 | 3 +/- 3 |
| 50 | 1.5 | 43 +/- 8.8 | 0 +/- 0 |
| 75 | 6.1 | 71 +/- 6.4 | 2 +/- 1 |

[a] Ice cream mixes were inoculated with specified amounts of lactase enzyme and stored 24 h at 4$^{o}$C for hydrolysis.
[b] Averages based on three replications.

**Table 2.** Attributes of ice cream with various degrees of lactose hydrolysis.

| Target lactose hydrolysis (%) | Average freezing point[1] ($^oC$) (mean +/- s.e.) | Rate of melting (mL/min) (mean +/- s.e.) | Firmness[2] (joules) (mean +/- s.e.) | Ease of dipping[3] |
|---|---|---|---|---|
| 0 | $-1.63^{a}$ +/- 0.01 | $1.43^{b}$ +/- 0.05 | $0.35^{a}$ +/- 0.03 | $3.33^{a}$ |
| 25 | $-1.64^{a}$ +/- 0.01 | $1.50^{ab}$ +/- 0.07 | $0.23^{b}$ +/- 0.03 | $2.56^{b}$ |
| 50 | $-1.67^{a}$ +/- 0.01 | $1.56^{a}$ +/- 0.01 | $0.14^{bc}$ +/- 0.01 | $2.31^{bc}$ |
| 75 | $-1.72^{b}$ +/- 0.01 | $1.67^{a}$ +/- 0.01 | $0.10^{c}$ +/- 0.01 | $1.79^{c}$ |

[1] Determined using a thermistor cryoscope.

[2] Measured as work (joules) between $-16.5^oC$ and $-18^oC$.

[3] Average rank on a four point scale (1 = easiest to dip and 4 = hardest to dip); [a, b, c, d] values designated with the same superscript are not significantly different ($p < 0.05$) as determined by Friedman's analysis.

[a, b, c, d] Values designated with the same letter within a column are not significantly different ($p<0.05$) as determined by ANOVA test.

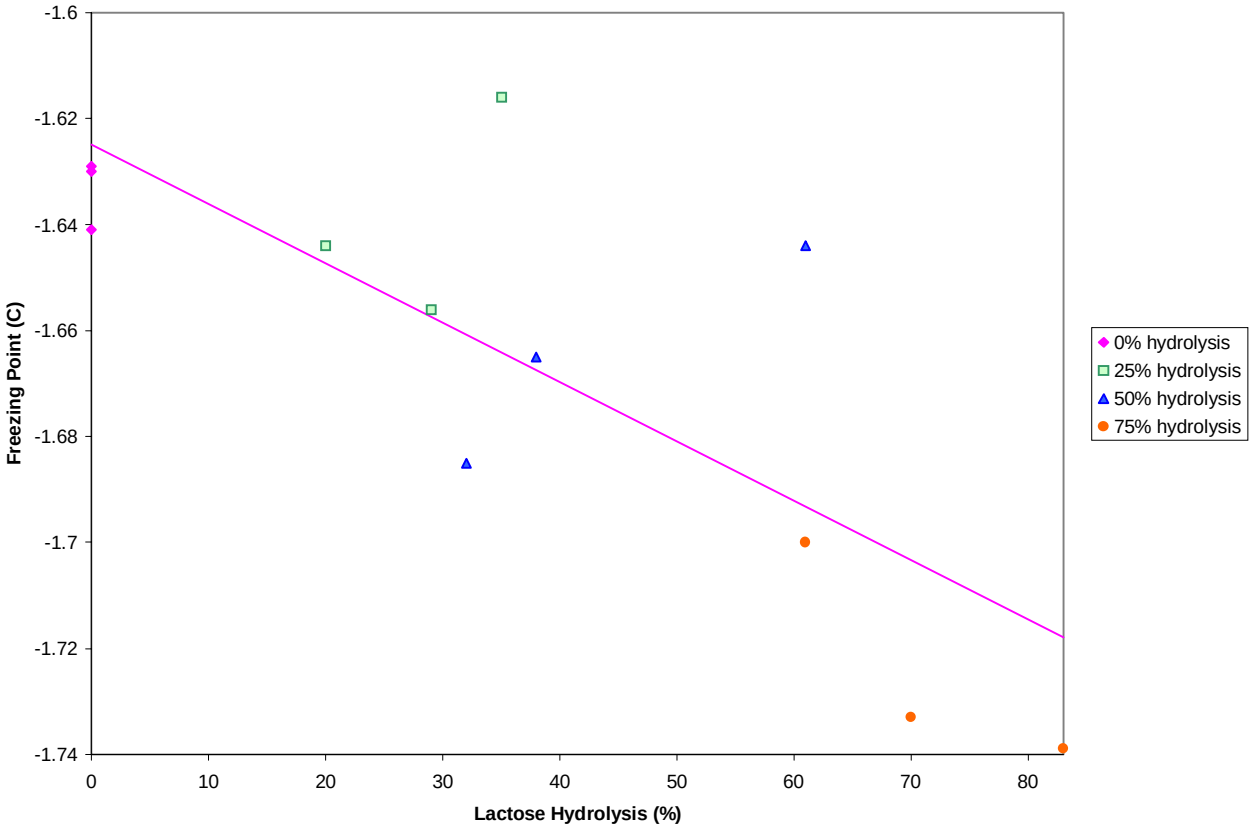


**Figure 1.** Relationship between freezing point and percent lactose hydrolysis in ice cream. ($Y = -1.62496 - 0.00112X$; $R^2 = 0.61$). Different symbols represent target hydrolysis levels. Three replications targeting these hydrolysis levels were completed

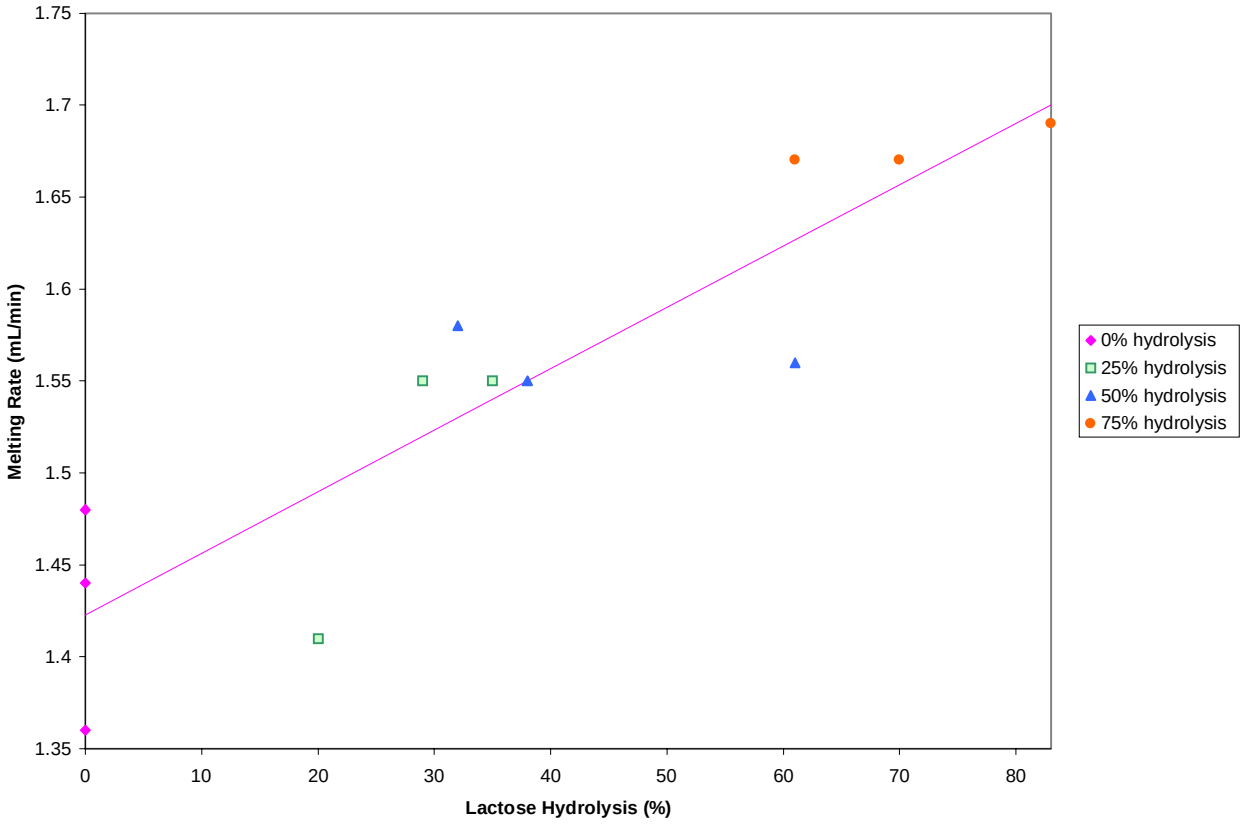


**Figure 2.** Relationship between melting rate and percent lactose in ice cream mix. (Y = 1.423738 + 0.003322X; $R^2 = 0.80$). Different symbols represent target hydrolysis levels. Three replications targeting these hydrolysis levels were completed.

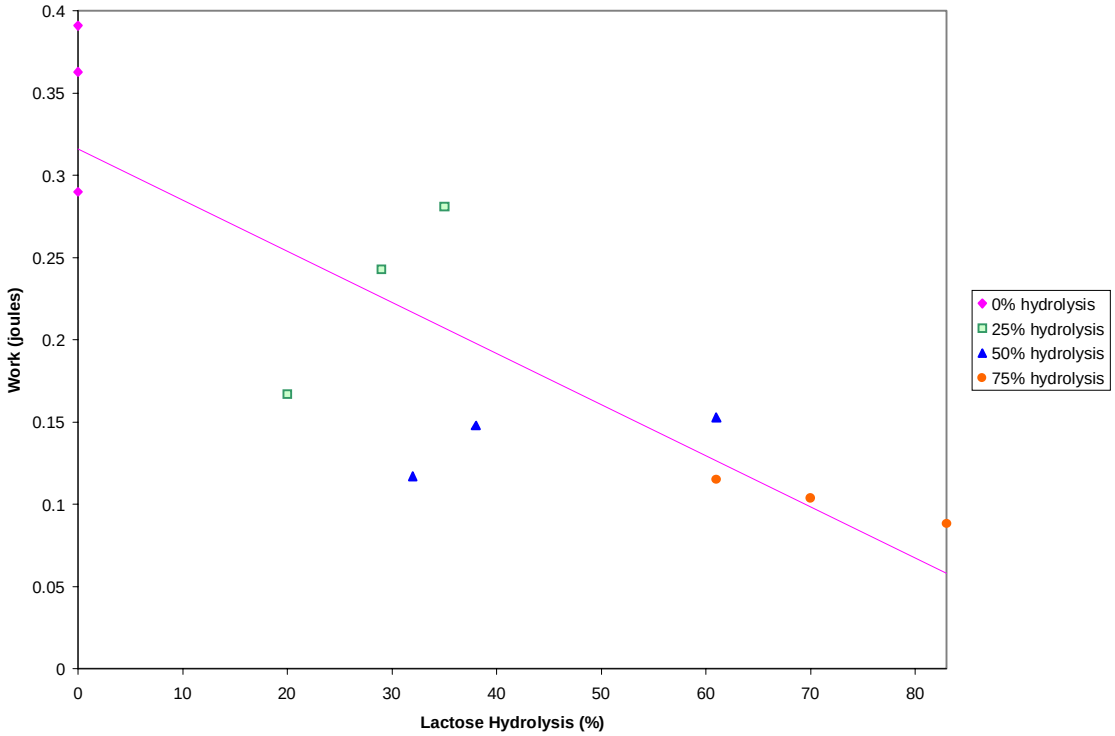


**Figure 3.** Relationship between firmness of ice cream and percent lactose hydrolysis of ice cream mix. ($Y = 0.316049 - 0.00311X$; $R^2 = 0.70$). Different symbols represent target hydrolysis levels. Three replications targeting these hydrolysis levels were completed.