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

Kristen Matak

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Committee Members:
Dr. Susan E. Duncan
Dr. James Wilson
Dr. Cameron. R. Hackney
Dr. Susan S. Sumner

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Written By
Kristen E. Matak

(ABSTRACT)

Two studies were conducted to examine the effects of lactose hydrolysis on freezing point and dipping characteristics of ice cream. The overall research objective was to determine changes in freezing point, texture and ease of dipping ice cream as a result of lactose hydrolysis. It was also the goal of this research to relate observations from the sensory dippability study with hardness and yield stress data to determine if the latter methods could be used as an alternative to human testing of dippability.

In the first experiment, 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% hydrolysis) 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.

In the second study, ice cream mixes were treated with lactase (EC 3.2.1.23) from the microbial sources Kluyveromyces lactis and Aspergillus oryzae to cause 0 to 100% lactose hydrolysis. Compression measurements and yield stress as measured by the vane method were both affected by the temperature of the samples. R^2 values for compression measurements as related to lactose hydrolysis were higher then those obtained for yield stress measurements. Human evaluation determined a difference (p < 0.05) between the control samples (0% hydrolyzed) and the treatment groups (80% and 100% hydrolyzed).

This research demonstrated a relationship between lactose hydrolysis and ease of dipping ice cream. The results implied that the effect of lactose hydrolysis on the dipping characteristics could be evaluated successfully by three different methods: the vane method, compression measurements, and human evaluation. Changes in freezing point due to lactose hydrolysis were minimal, implying that monitoring freezing point is not enough to determine textural characteristics.
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CHAPTER I
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 in the seventies (Anon., 1984). The cost of the enzymes is 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).

When lactose is hydrolyzed, enzymes are used to break down the disaccharide into its constituent monosaccharides; D-glucose and D-galactose. This reaction allows most consumers who are lactose intolerant to comfortably ingest products that contain the milk sugar. Lactose hydrolysis also results in an increased amount of soluble solutes in solution which lowers freezing point (Iversen, 1983). Depressing the freezing point of ice cream mix affects textural characteristics of ice cream (Lindamood et al., 1989; Guy, 1980).

Relative sweetness of lactose is approximately one-fifth that of sucrose (Fennema, 1996). 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. The increase in dissolved solutes may influence the characteristics of frozen ice cream mixture by depressing the freezing point, increasing the relative sweetness, and promoting the ease of dippability of frozen ice cream (Iversen, 1983). A relationship between ice cream hardness and calculated freezing point depression was established by Lindamood (1989) and it was implied that the hydrolysis of sucrose and lactose in the mix may alter the properties of ice cream toward a more "dippable" product (Lindamood et al. 1989).

The overall research objective of this project was to determine changes in freezing point, texture, sensory characteristics, and ease of dipping ice cream as a result of lactose hydrolysis. Lactose hydrolysis was accomplished by lactase enzymes from two microbial sources. Enzyme preparations from Aspergillus oryzae (fungal source) and Kluyveromyces lactis (yeast source) were utilized to achieve 0%, 25%, 50%, 75%, 80% and 100% lactose hydrolysis in ice cream samples. It was also the goal of this research to relate ratings from the sensory dippability study with compression and yield stress data to determine if these methods may be used as an alternative to human testing of dippability.
CHAPTER II
REVIEW OF LITERATURE

A. Review of Lactose Hydrolysis and Enzyme Activity

Lactose and Lactose Intolerance. Lactose (4-ortho-β-D-galactopyranosyl-D-glucose) is a disaccharide that is composed of the two simple sugars D-glucose and D-galactose (Fennema, 1996; Geilman, 1993). It is the principal carbohydrate and the natural sweetener of mammalian milk (Houts, 1988; Holsinger and Kligerman, 1991). Lactose contributes up to 40% of the energy consumed by human infants during nursing (Fennema, 1996). In order to utilize this energy, the lactose must be hydrolyzed to the constituent monosaccharides. In its unaltered state, lactose is indigestible by an estimated 70% of the world’s population (Holsinger and Kligerman, 1991). Studies documented by Houts (1988) showed that among the U.S. population, American whites were estimated to have the lowest prevalence of lactase deficiency (6-25%) while American blacks and Mexican-Americans showed much higher rates (45-81% and 47-74%, respectively). The prevalence of lactose intolerance has also been found to increase with age (Reiter, 1991). The growing number of ethnic groups and the aging of the U.S. population will increase the number of people experiencing lactose intolerance. This growth provides a potential market for food products in which the lactose has already been hydrolyzed.

Lactose Hydrolysis. Evolution of lactose-reduced products began when the enzyme β-galactosidase (lactase) became accessible to the consumer. The consumer could add this enzyme to a glass of milk to hydrolyze the lactose. Most consumers could then ingest the "lactose-reduced" milk without the discomfort associated with lactase deficiency (Anon., 1984). With improvements in processing techniques, hydrolyzing the lactose before packaging certain dairy products has become more prevalent (Anon., 1984). Through research, the procedure has become more cost effective due to increased understanding of the process and its effects on product characteristics (Holsinger and Kligerman, 1991). With these new advancements, various types of lactose-reduced foods such as milk, American cheese, cottage cheese and ice cream are making their way into this growing market (Reiter, 1991).

Many research efforts have been focused on ways to reduce or remove the lactose in dairy products (Kocak and Zadow, 1989). One of the more common ways to accomplish this is through the use of enzymes. Three techniques may be utilized to accomplish enzymatic lactose hydrolysis: (1) "single-use" or "throw-away" batch systems; (2) recovery systems (lactase re-use systems); and (3) immobilized enzymes which are systems where the enzyme is chemically bound to an inert matrix (Holsinger and Kligerman, 1991; Zadow, 1986). It has been shown by USDA that the "single-use" enzyme batch system could be used conveniently during the development of new lactose-reduced products, whereas the latter systems are better for large-scale operations (Holsinger and Kligerman, 1991). The method that is ultimately used is dependent on a number of factors including pH of the product, maximum temperature the product will reach and how long it will remain there, contact time, activity of the enzyme, substrate, and cost (Zadow, 1986).

Measurement of Lactose Hydrolysis and Enzymatic Activity. There are two main methods of determining the amount of lactose (or degree of hydrolysis) that is present: enzymatic and chromatographic. Chromatographic measurements have been
used for carbohydrate analysis to provide quantitative data for such sugars as fructose, glucose, galactose, sucrose, maltose, lactose, and higher sugars (Arbuckle, 1986). Chromatographic methods are very specific because they allow for multiple low-molecular weight carbohydrates to be analyzed consecutively (Mustranta and Ostman, 1997). However, they are complicated and require high performance liquid chromatography instrumentation or thin layer chromatographic techniques; therefore the enzymatic approach often is preferred because it is sensitive and easy to perform (Mustranta and Ostman, 1997).

To determine the extent of lactose hydrolysis using the enzymatic method, D-galactose is oxidized by nicotinamide-adenine dinucleotide (NAD) to form D-galactonic acid in the presence of the enzyme β-galactose dehydrogenase (Gal DH) (Mustranta and Ostman, 1997). The amount of NADH formed as a result of this reaction is stoichiometric to the amount of lactose and D-galactose. The amount of NADH may be quantitatively determined by measuring the light absorbency of the solution at 340 nm and then converted, mathematically, to determine the concentration (g/100 mL) of lactose and D-galactose (Mustranta and Ostman, 1997).

Enzymatic 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-nitrophenol released from this reaction is measured spectrophotometrically and the lactase activity is estimated as the amount of enzyme which liberated one μmole o-nitrophenol from ONPG per minute per gram samples at 37°C (Shah and Jelen, 1990).

Enzymes used as a treatment for hydrolysis of lactose may be extracted from a number of animal, plant, and microbial sources; depending on the source, the properties could differ considerably (Cavaille and Combes, 1995). Those enzymes from microbial sources are of technological interest because they produce high yields of enzyme (Cavaille and Combes, 1995). Lactase preparations usually fall into one of two groups: "acid" or "neutral" lactases (Jelen, 1993). Acid lactases are characterized by a pH optimum around 4.5 and a temperature optimum of 55°C, whereas neutral lactases show an activity optimum between pH 6.0 and 6.5 and temperature optimum between 36 and 38°C (Jelen, 1993).

Microbial sources will allow large quantities of suitable enzymes to be extracted (Palmer, 1985). Guy and Bingham (1978) examined the activity of lactase (Saccharomyces lactis) added to skim milk and whey to determine optimum conditions for the hydrolysis of lactose. Addition of the enzyme was accomplished by adding it directly to pre-tempered skim milk and whey at pH 6.6. Lactose in unconcentrated skim milk was hydrolyzed at a faster rate (15%) than for milk whey (Guy and Bingham, 1978). Optimal pH was 6.5, the normal pH of milk. The enzyme was most active at temperatures below 50°C. Heating the enzyme drastically reduced the activity level (Guy and Bingham, 1978). This particular enzyme would be classified as a "neutral" lactase.

Two enzyme sources of particular importance for this project were Aspergillus oryzae and Kluyveromyces lactis. These fall into the acid and neutral groups, respectively. Aspergillus oryzae is commonly used to obtain fungal enzyme, an acid lactase, with a pH optimum between 4.0 and 6.0 and a temperature optimum between 50 and 60°C (Jelen, 1993). 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 and is considered a neutral lactase. 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 purification is important to ensure that no unwanted reactions occur (Palmer, 1985). Lindamood et al. (1989) conducted a study to determine the effects of lactose and sucrose hydrolysis in ice cream samples. The changes in texture, firmness (hardness), freezing point, melting characteristics and relative sweetness were assessed. The enzyme utilized for lactose hydrolysis was a commercially available lactase (Lactaid brand, McNeil Specialties, Inc. Pleasantville, N.J.). In ice cream mixes treated with this enzyme only, a significant amount of sucrose hydrolysis also occurred. After further analysis it was determined that the sucrose had hydrolyzed at a faster rate than the lactose. The batch with a target level of 25% lactose hydrolysis was determined to have 22.4% lactose hydrolysis and 15.1% sucrose hydrolysis. The batches with target levels of 50% and 100% lactose hydrolysis were shown to have 44.0% and 78.4% lactose hydrolysis, respectively, and 7.6% and 22.5% sucrose hydrolysis, respectively. It was concluded that the lactase enzyme preparation was impure.

B. Implications of Lactose Hydrolysis on Ice Cream

The dairy industry is expanding their product lines to include more specialty items to meet consumer needs and expectations. Success of these items depend on the results of extensive testing and numerous reformulations (Anon., 1984).

Ice cream is a popular dairy dessert made by freezing a pasteurized mixture composed of about 0-20% fat, 8-15% milk solids not fat (MSNF), 13-20% sugar, 0-0.7% stabilizer and emulsifier, and 36-43% total food solids (Marshall and Arbuckle, 1996). Lactose makes up over one third of the solid matter in milk, and approximately 20% of the carbohydrate content in ice cream (Marshall and Arbuck, 1996). The percentage of lactose in ice cream is dependent on the formulation, including the amount of MSNF and fat, in the mixture. Superpremium types of ice cream have a higher percentage of fat and the source of MSNF are usually limited to skim milk solids. These contain about 50% less lactose than whey solids which are 72% lactose (Marshall and Arbuckle, 1996). The estimated percentage of lactose in vanilla ice cream is 6-7% (Carper, 1986). The effects that lactose hydrolysis has on the freezing point, texture, and sensory characteristics of ice cream are discussed below.

Freezing Point. Freezing point of an ice cream mixture is directly proportional to the number of particles in solution (Iversen, 1983; Mitchell, 1989). The more solids dissolved in solution, the lower the freezing point. The freezing point varies with the composition of the mix and concentration of the soluble constituents within the mix (Marshall and Arbuckle, 1996).

Freezing point is influenced by the major components of low molecular weight; i.e. milk salts, sugar, corn syrup solids, and milk sugar (Kilara, 1997; Mitchell, 1989). The principal constituents responsible for freezing point depression are milk sugar (lactose) and salts (Mitchell, 1989). Lactose makes up 54.5% of the serum solids in ice cream mix and the degree that lactose depresses freezing point is the same as that caused by sucrose (Table 1)(Kilara, 1997; Smith and Bradley, 1983). The contribution of corn syrup solids to freezing point depression depend on the dextrose equivalency (DE) of these solids, the higher the DE, the larger the freezing point depression factor (Table 1).
Variations of fat globules, protein, emulsifiers, and stabilizers have been shown to have no significant effect on the freezing point (Jaskulka et al., 1993), therefore, the contribution of sugars to the freezing point are calculated by the following formula by Kilara (1997):

\[
\% \text{ sucrose} + \% \text{ lactose} + \% \text{ sucrose equivalents} \times 100\%
\]

\[
\% \text{ water in the mix}
\]

The effects of milk salts on freezing point depression are calculated by the formula as follows (Kilara, 1997):

\[
\% \text{ serum solids} \times 2.37 \times 100
\]

\[
\% \text{ water}
\]

The freezing point of the mix can be computed by the known concentration of sugars in the solution and the molecular weight of the sugars (Marshall and Arbuckle, 1996). Sugars with high molecular weights will cause the least lowering of freezing point, while sugars with the low molecular weights will have the greatest effect on freezing point (Marshall and Arbuckle, 1996).

Iversen (1983) established that it is the molar concentration of the sugars that determine freezing point of ice cream. Ice cream mixes with high sugar and MSNF content will have a lower freezing point than mixes with high fat, low MSNF, or low sugar content (Marshall and Arbuckle, 1996).

Typical ice cream mix containing 12% fat, 11% MSNF, 15% sugar, 0.3% stabilizer, and 61.7% water has a freezing point of approximately -2.5°C (Marshall and Arbuckle, 1996). Lindamood et al. (1989) calculated the freezing point based on molar concentration of sugars (Iversen 1983) and determined that as the degree of lactose hydrolysis in the ice cream samples increased, the freezing point was comparatively depressed. The freezing point of a non-treated ice cream sample (10% milkfat, 12% MSNF, 12% sucrose, 5% corn syrup solids and 0.25% stabilizer-emulsifier blend) was calculated as -1.45°C. The samples with 25%, 50% and 100% lactase treatment (7.9 mL enzyme/100 L mix) had calculated freezing points of -1.62°C, -1.67°C, and -1.92°C, respectively (Lindamood et al., 1989). Lindamood et al. (1989) concluded that the more hydrolysis that takes place in the product, the lower the freezing point.

Guy (1980) evaluated the effects of using either 67% or 79% lactose hydrolyzed sweet whey solids (LHW) as a replacement for both MSNF and cane sugar. The point at which the mix was frozen was judged by an experienced operator and the freezing temperature was taken. The mix formulated with no extra whey solids had a freezing temperature of -4.5°C. The freezing temperatures of the mixes formulated with 11% whey solids from 67% LHW and 79% LHW were -5.0°C and -6.0°C, respectively (Guy, 1980). Guy (1980) attributed the change in freezing temperatures to increased levels of salts and monosaccharides from LHW.

Freezing point of milk is determined using a thermistor cryoscope which measures temperatures on the Hortvet (H) scale. Conversion of Hortvet to Celsius is: \(°H = 1.03916°C + 0.00250 \) or \(°C = 0.96231°H - 0.00240 \), where both readings have negative values (Marshall, 1993). The cryoscope is calibrated using 7% and 10% NaCl solutions which have freezing points of -0.422°C (-0.408°C) and -0.621°C (-0.600°C), respectively (Marshall, 1993). Determination of the freezing point of ice cream mixtures using a thermistor cryoscope requires a 1:4 dilution of the mix. Ohmes et al. (1998) diluted ice cream mixtures with distilled water (1:4 dilution) and measured freezing points (in
degrees Hortvet) using a thermistor cryoscope. An equation was used to convert degrees Hortvet to degrees Celsius: °C = 0.9(°H - 0.0024)(dilution factor).

Baer et al. (1980) demonstrated that there was a direct relationship between the degree of lactose hydrolysis and freezing point depression in acid whey and lactose solutions. Acid whey solutions (with known concentrations of lactose) and 5% lactose solutions were inoculated with lactase (20,000 ONPG/g). For each 1% lactose hydrolyzed in the acid whey, the freezing point was depressed 0.0510°H (0.03774°C)(3.0% standard error) and for each 1% lactose hydrolyzed in the 5% lactose solution, the freezing point was depressed 0.0486°H (0.03758°C) (1.1% standard error). Since the freezing point was depressed 0.050°H (0.04284°C) for each 1% reduction in lactose concentration, the percent lactose hydrolysis could be calculated from the initial lactose concentration and freezing point of a non-hydrolyzed sample, and the subsequent freezing point. Baer et al. (1980) concluded that this method could provide a fast, reliable estimation of the amount of lactose hydrolysis in whey and similar products.

Rapid melting of ice cream samples is a desirable characteristic and is associated with softer, extrudable products (Lindamood et al., 1989). Low freezing point is often responsible for accelerated melting (Marshall and Arbuckle, 1996). A quality ice cream should demonstrate certain melting properties. Desirable melting qualities include initiation of melting within 10-15 minutes of dipping and a melted product forming a homogenous liquid with little or no foam (Marshall and Arbuckle, 1996). Lindamood et al. (1989) reported melting characteristics of ice cream samples with lowered freezing points as assessed by a single trained panelist. A weak relationship between the melting rate (mL/min) and degree of hydrolysis was determined (Lindamood et al., 1989). In a non-hydrolyzed ice cream sample the rate of melting was 0.45 +/- 0.13 mL/min. In samples treated with 25%, 50%, and 100% lactase (7.9 mL enzyme/100 L mix) the melting rates were determined to be 0.32 +/- 0.02, 0.82 +/- 0.30, and 0.81 +/- 0.07, respectively. Guy (1980) evaluated the melt-down of ice cream samples that contained either 67% or 79% LHW. Melting resistance was determined as the weight percent of ice cream melted in 90 min at 37°C. The percentage of melt-down from ice creams containing LWH were not significantly different from the control. Lindamood et al. (1989) concluded that hydrolysis of lactose in ice cream does not create melt-down to an extent which might be helpful in extrusion and dipping at low temperatures.

**Textural Characteristics.** The more lactose that is present in the mix, the higher the risk of developing a sandy texture due to lactose crystallization in the final product (Marshall, 1991). This defect is caused by the supersaturation of lactose in the unfrozen phase of the mixture and fluctuating storage temperatures (Livney et al., 1995). The formation of crystals begins when the storage temperature of the frozen dessert increases, allowing the lactose molecules to change from their original chaotic state to organized nuclei (Marshall, 1991). These first crystals serve as a location to which other lactose molecules can easily attach during the next temperature increase (Marshall, 1991). The result of this activity is the formation of crystals which give ice cream a sandy or coarse texture. The hydrolysis of lactose in ice cream results in a smoother texture (Marshall and Arbuckle, 1996). By hydrolyzing the lactose in the ice cream mixture, the total amount of the disaccharide is reduced considerably, which may result in a mixture that is less prone to crystallization.
Textural characteristics of ice cream are of critical importance during the physical act of scooping, or "dipping", the ice cream out of its original container (Marshall and Arbuckle, 1996). Dipping losses are affected by loss of air, ice cream structure, dipping temperature, overrun, and mix composition. Two important elements that influence the volume of hand-dipped ice cream are: (1) resistance offered by the ice cream, which prevents air from being compressed or expelled; and (2) amount of force required to push the dipper into the ice cream (Marshall and Arbuckle, 1996). Overrun, the incorporation of air into the mix, facilitates the ease of dipping (Iversen, 1983). The more air whipped into the product decreases the resistance offered by the ice cream which results in a product that is easier to dip (Arbuckle, 1996). Consistency and storage stability of the frozen ice cream may be adversely affected when the overrun is increased to a point above which is appropriate (Iversen, 1983). The proper way to dip ice cream is to move the sharp-edged dipper (ice cream scooper) in a circular manner around the canister cutting off ribbons of ice cream to form a smooth round ball (Marshall and Arbuckle, 1996). The ice cream should not be compressed. Appropriate dipping technique optimizes the number of ice cream scoops and limits volume loss (Marshall and Arbuckle, 1996).

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). Hardness of frozen ice cream is important when transferring ice cream from one container to another, as occurs during dipping. Ease of dipping can be evaluated by a human panel; textural parameters, such as firmness, can be measured mechanically. Physical measurements of firmness and yield stress have been used to estimate the ease of dipping ice cream (Lindamood et al., 1989; Briggs et al., 1996). Lindamood et al. (1989) evaluated the firmness of ice cream samples treated with 0%, 25%, 50% and 100% lactase (7.9 mL enzyme /100L mix) using a universal testing machine. A pre-cooled cylindrical probe (7.00 mm diameter) mounted on a 5 kg weigh beam (Instron, Model 100, Canton, MA) was used to take compression measurements on ice cream samples maintained at a temperature of -15°C. The speed of the crosshead was set at 20 mm/min and depressed 10 mm into the sample. 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 established that some sucrose hydrolysis accompanied the hydrolysis of lactose.

The same test was run for samples treated with invertase, to hydrolyze sucrose, and a combination of invertase and lactase (Lindamood et al., 1989). The relative firmness of the samples with targeted levels of 25% and 50% sucrose hydrolysis, and samples with a combination of 25% sucrose and 25% lactose hydrolysis were determined not to be different (p > 0.05) from the control (0.42 J, 0.33 J and 0.34 J, respectively). However, a statistical difference was shown between the control and the samples with 100% sucrose hydrolysis having a relative firmness of 0.26 J, and the samples with targeted 50% and 100% sucrose and lactose hydrolysis having a relative firmness of 0.18 J and 0.13 J, respectively. A relationship between ice cream firmness and calculated
freezing point depression was established and it was implied that the hydrolysis of sucrose and lactose in the mix may alter the properties of ice cream toward a more "dippable" product (Lindamood et al. 1989).

Guy (1980) evaluated firmness in ice creams that contained either 67 or 79% LHW by using a penetrometer. Five-second penetrations were taken in triplicate. Firmness decreased significantly with increased percentage of LHW in the formulation.

Ease of dippability of a finished ice cream product can also be estimated using the vane method to determine yield stress, a term used to describe the minimum stress required to cause flow (Steffe, 1992). Yield stress is important in food applications such as sensory perception ("mouthfeel"), thickness after dip coating (leveling and sagging of a chocolate coating), mechanical spreading (butter, ketchup, mayonnaise), ability to hold structure (whipped topping), and performance of processing equipment (Steffe, 1992).

The vane method was originated to provide a means for the direct measurement of the true yield stress of concentrated suspensions under static conditions (Dzuy and Boger, 1983). Dzuy and Boger (1983) offered two advantages of the vane method in the measurement of yield stress: (1) the vane allows the material to yield under static conditions and within the material itself, and (2) the introduction of the vane into the suspension does not cause any significant disturbance to the sample prior to measurement. The vane method is based on the measurement of the yielding moment when the torque exerted on a vane with a small number (usually 2-8) of blades, arranged at equal angles around a small cylindrical shaft, reaches a maximum value (Dzuy and Boger, 1983 and 1985). The recorded torque data is divided by the surface area of the cylindrical volume defined by the outer edges of the rotating vane fixture to determine the magnitude of yield stress (Wilson et al., 1993). Steffe (1992) suggested that the dimensions for the vane stay within the following limits: \( D/d > 2.0; Z_1/d > 1.0; Z_2/d > 0.5 \), where \( D \) is the diameter of the sample vessel, \( d \) is the diameter of the vane, \( Z_1 \) is the depth the vane shaft is submerged in the sample, and \( Z_2 \) is the distance from the bottom of the vane to the bottom of the sample. Briggs et al. (1996) demonstrated that this method is advantageous for testing the physical characteristics of frozen ice cream because it does not destroy the product structure during sample loading and allows the original container to be the sample vessel.

The principle of the vane method is centered on extremely slow shearing to detect the yielding of material (Dzuy and Boger, 1983). Rotating the vane at low speeds is required to achieve satisfactory yield stress measurements (Dzuy and Boger, 1983). Dzuy and Boger (1983) determined that at high rotational speeds (\( x > 8 \) rpm), significant viscous resistance together with instrument inertia and insufficient damping may introduce errors to the measured maximum torque and hence to the calculated yield stress of bauxite residue suspensions (red mud). From these results it was determined that the suitable operating range of vane rotational speeds for red mud should be from 0.1 to 8 rpm (Dzuy and Boger, 1983). The effect of vane dimensions on measured yield stress were determined using three vanes of different length-to-diameter ratios (1.48, 0.95, and 1.92, respectively). Two of the vanes had almost equal lengths but different diameters and two vanes had the same diameter but different lengths. The vanes were rotated at 0.1 rpm in red mud samples at different concentrations. Dzuy and Boger (1983) reported that the differences in the yield values were within the experimental error and the effect due to vane dimensions can be neglected for red mud.
Briggs et al. (1996) suggested that the ability of ice cream to be dipped is a direct consequence of yield stress. They assessed the feasibility of the vane method to measure yield stress in ice cream and to determine how yield stress was affected by temperature. This study represented the first use of the vane method for measurements of the viscosity of ice cream. Yield stress measurements of two commercially available brands of vanilla and chocolate ice cream were compared. The fat content of the two brands of vanilla ice cream were 11.8% and 10.9% and the percentage of total solids were 39.7 and 41.1, respectively. The fat content of the two brands of chocolate ice cream were 10.9% and 7.8% and total solids for each brand was 35.9%. The ice cream was stored at a temperature range of -16°C to -12°C for at least 24 h prior to testing in 1.89 L cartons. The vane test instrument was maintained in a room at approximately 22°C. A carton of ice cream was placed into the sample holder and the vane (4 bladed; height = 3.8 cm; diameter = 1.8 cm) was immersed completely into the sample by adjusting the vertical track to a penetration depth of 5.6 cm (the vane height plus the vane diameter). Once the vane was positioned, the sample was rotated (speed = 1 rpm) and the torque on the vane was measured as a function of time. The yield stress was calculated from the maximum torque and vane dimensions. The results of this study showed that, upon rotation of the sample, the torque on the vane increased until a peak torque was achieved, followed by a gradual decrease in torque. Yield stress was exceeded at the peak torque, and torque decreased as the material structure was broken and flow began. Briggs et al. (1996) concluded that the results of this research demonstrated the viability of the vane method as a means of measuring the yield stress of ice cream. Although no correlation was made, they suggested that yield stress measurement may be correlated to body, texture and dippability, and that it has been shown to offer an objective way to evaluate the flow behavior of frozen desserts. This method may be applied in the industry for quality control, and research and development (Briggs et al., 1996). The vane method produces results that are comparable with other techniques measuring yield stress and have been employed to calculate the yield stress of food suspensions such as melted chocolate, mayonnaise, tomato concentrates, and salad dressing (Briggs et al., 1996).

**Sensory Characteristics.** Published reports relating human perception of dippability of ice cream to degree of lactose hydrolysis or instrument measurements of ice cream firmness have not been found. It is possible that such information exists within independent companies. It is recognized that Good Humor Breyers (Green Bay, WI) manufactures a softer hard pack ice cream, Breyers Soft and Creamy; based on a review of the ingredient statement and personal communication with Jodi Elling, Food Technologist at Good Humor Breyers (1998), this is possible by increasing the level of monosaccharides in the formulation.

Relative sweetness is an arbitrary analysis in which there are no chemical tests to scientifically determine the characteristic (Marshall and Arbuckle, 1996). Relative sweetness is determined by comparing the sweetener in question to that of sucrose, which is given the value of 100. Lactose constitutes over one-third of the solid matter in milk, and depending on the composition of the mix, makes up approximately one-fifth of the carbohydrate in ice cream (Marshall and Arbuckle, 1996; Fennema, 1996). Lactose is also about one-fifth as sweet as sucrose and is shown to function as a body-building agent and freezing point adjuster in ice cream (Fennema, 1996; Marshall, 1991). According to
Zadow (1986), hydrolysis of 70% of the lactose in milk increases its sweetness by an amount comparable to the addition of about 2% sucrose.

Hydrolysis of lactose to its component monosaccharides results in a sweeter milk (Zadow, 1986). Sweetness perception tests conducted by Sutton et al. (1996) on 70% and 90% lactose-reduced milk and custard samples compared to non-hydrolyzed milk and custards, respectively, indicated that there was an observable difference in sweetness, based on ranking tests, between the control and hydrolyzed milk samples but not among the custard samples. The results of the ranking tests indicated that the reduction of lactose in milk samples did affect sweetness perception of milk. The perceptible increase in sweetness of the lactose-reduced milk samples was due to the naturally bland flavor of milk (Sutton et al., 1996). The same simple ranking test was run for custard samples which had 89.3% hydrolysis of lactose yet compared to non-hydrolyzed custards a perceptible increase in sweetness was not found (Sutton et al., 1996). These results imply that since custard has a high concentration of sugars and is more complex than milk, the compositional differences may affect sweetness perceptions (Sutton et al., 1996).

Lindamood et al. (1989) calculated relative sweetness for ice cream samples treated with lactase, invertase, and a combination of both enzymes using the relative sweetness values for different sugars reported by Iversen (1983). It was determined that the relative sweetness of the sample with the most amount of hydrolysis was increased 134% of the control. Twenty-five untrained panelists and 3 trained panelists rated their preference (acceptability) of the ice cream samples using a 9-point hedonic scale. Although the calculated relative sweetness was much more than the control, neither group of judges complained that the samples were excessively sweet (Lindamood et al., 1989). These results imply that the perception of sweetness in lactose-reduced frozen ice cream samples should not be different than that of the original product. Ice cream should have enough sugars from other sources to mask any change in sweetness brought about by the hydrolysis of lactose.
REFERENCES

Elling, J. 1995. Personal Communications. Food Technologist at Good Humor Breyers, Green Bay, WI.
Table 1. Freezing point depression factors caused by sweeteners (Smith and Bradley, 1983).

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Molecular Weight</th>
<th>Freezing Point Depression Factor</th>
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<tr>
<td><strong>Disaccharides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
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<td>Lactose</td>
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<td>1.00</td>
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<tr>
<td>Maltose</td>
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<td>0.98</td>
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<td><strong>Monosaccharides</strong></td>
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</tr>
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<td>Galactose</td>
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<td>1.92</td>
</tr>
<tr>
<td>Fructose</td>
<td>180</td>
<td>1.92</td>
</tr>
<tr>
<td><strong>High Fructose Corn Syrups</strong></td>
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<tr>
<td>42% Fructose</td>
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<td>55% Fructose</td>
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</tr>
<tr>
<td>90% Fructose</td>
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</tr>
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<td><strong>Corn Syrup Solids</strong></td>
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<td>10 DE Maltodextrin</td>
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<td>0.21</td>
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<tr>
<td>36 DE Corn Syrup</td>
<td>543 (472)*</td>
<td>0.64 (0.725)*</td>
</tr>
<tr>
<td>42 DE Corn Syrup</td>
<td>428</td>
<td>0.80 (0.799)*</td>
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<tr>
<td>52 DE Corn Syrup</td>
<td>(360)*</td>
<td>(0.950)*</td>
</tr>
<tr>
<td>64 DE Corn Syrup</td>
<td>296 (290)*</td>
<td>1.15 (1.179)*</td>
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<td>Xylitol</td>
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*Values in parentheses provided by (Tharp, 1981)