CHAPTER IV

PHYSICAL AND SENSORY MEASUREMENTS OF DIPPING CHARACTERISTICS OF LACTOSE-HYDROLYZED ICE CREAM

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ABSTRACT

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. Human evaluation determined a difference ($p < 0.05$) between the control samples (0% hydrolyzed) and the treatment groups (80% and 100% hydrolyzed).

Key Words: dippability, ice cream, lactose hydrolysis, vane method

INTRODUCTION

The dairy industry is expanding their product lines to include more specialty items to meet consumer needs and expectations. Extrudable ice creams that are easier to dip at home freezer temperatures are some of the new novelty frozen desserts (Lindamood, 1989). Dipping characteristic may be enhanced by: 1) depressing the freezing point, 2) increasing the air whipped into the product, or 3) temporarily increasing the storage temperature (Lindamood, 1989). In commercial products, improved dipping characteristics have been obtained primarily by freezing point depression (Lindamood, 1989).

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 the 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). Variations of fat globules, protein, emulsifiers, and stabilizers have no significant effect on the freezing point (Jaskulka et al., 1993).

Lactose makes up over one third of the solid matter in milk, and approximately 20% of the carbohydrate in ice cream (Marshall and Arbuckle, 1996). Lactose in ice cream mix is from milk and milk solids not fat (MSNF). The percentage of lactose in ice cream is dependent on the amount of MSNF and fat in the mixture. When the enzyme β-galactosidase (EC 3.2.1.23) is in the presence of lactose it acts as a catalyst to hydrolyze the lactose to D-glucose and D-galactose, thus increasing the total solids in solution without contributing to the caloric content of the mix (Bakken et al., 1992).

The hardness of frozen ice cream is important when transferring the ice cream from one container to another, as occurs during dipping. Textural parameters of firmness and yield stress have been measured mechanically and 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 0%, 25%, 50% and 100% lactose-hydrolyzed ice cream samples using compression measurements from a universal testing machine (Instron Model 100, Instron, Canton, MA). Non-hydrolyzed ice cream samples had a relative firmness of 0.44 J. The lactose hydrolyzed samples (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). Some sucrose hydrolysis accompanied the hydrolysis of lactose, contributing to freezing point depression and possibly affecting firmness of the product. A relationship between ice cream hardness and calculated freezing point depression was established and it was implied that the hydrolysis of sucrose and lactose in the mix altered the properties of ice cream toward a more "dippable" product (Lindamood et al. 1989). Human assessment of dipping characteristics of lactose hydrolyzed ice creams was not completed.

Ease of dippability of frozen ice cream has also been 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 significant 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 principle of the vane method originated to provide a means for the direct measurement of

the true yield stress of concentrated suspensions under static conditions (Dzuy and Boger, 1983). Briggs et al. (1996) suggested that the ability of ice cream to be dipped is a direct consequence of yield stress. 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). 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; 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).

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 whereas high rotational speeds ($x > 8$ rpm) may introduce errors to the measured maximum torque and hence to the calculated yield stress. This may result from significant viscous resistance together with instrument inertia and insufficient damping (Dzuy and Boger, 1983). The suitable operating range of vane rotational speeds for red mud were determined to be from 0.1 to 8 rpm (Dzuy and Boger, 1983).

Briggs et al. (1996) assessed the feasibility of the vane method to measure yield stress in ice cream. 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. Upon rotation of the sample (1 rpm), 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 demonstrated the viability of the vane method as a means of measuring the yield stress of ice cream. Although no correlation was made, Briggs et al. (1996) 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 overall research objective of this project 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 the firmness and yield stress data to determine if these methods may be used as an alternative to human testing of dippability.

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% sugar, and 0.3% stabilizer. The mix was stored at 4°C until it was frozen.

Lactose Hydrolysis of Ice Cream Mix

Lactose hydrolysis was accomplished by enzyme preparations from *Aspergillus oryzae* (DP432 Lot#970910-01, Valley Research, South Bend, IN) and *Kluyveromyces lactis* (Validase Yeast Lactase Lot #CQ7231, Valley Research, South Bend, IN). Enzyme preparations were stored at 4°C until addition to the ice cream mix. Enzyme preparations were filtered through sterile 0.45 μm acrodiscs (Gelman Sciences prod. no 4184, Fisher Scientific, Pittsburgh, PA) and added to 2.3 L (0.5 gal) of mix to achieve 100% hydrolysis after 24 h at 4°C.

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 levels of the yeast and fungal enzyme were 53,000 ONPG and 30,000 ONPG, respectively.

Preliminary testing determined that the addition of 2.5 mL yeast enzyme and 13 mL fungal enzyme to 2.3 L mix for 24 h at 4°C were appropriate levels to accomplish 100% lactose hydrolysis. These levels were determined by inoculating 1 L of mix with predetermined levels of enzyme and then testing the amount of lactose hydrolysis achieved after 24 h.

Enzyme-inoculated ice cream mixes were stored for 24 h at 4°C and shaken vigorously by hand for one minute at two intervals during the storage period to evenly 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 Boehringer Mannheim test kit information (Sucrose/D-Glucose test kit, Boehringer Mannheim, Indianapolis) for lactose and sucrose assays, respectively.

The hydrolyzed mix was then added to non-hydrolyzed mix immediately before freezing to achieve the desired level of hydrolysis (0%, 80% and 100% for the yeast enzyme; and 0% and 75% for the fungal). The degree of lactose hydrolysis was assessed again after the samples were frozen.

Freezing the Ice Cream Mix

Ice cream mixes (0%, 75%, 80%, and 100% hydrolysis from each enzyme source) 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 of the mix was determined using a thermistor cryoscope (Advanced Milk Cryoscope Model 4C, Advanced Instrument Inc., MA) and recorded in degree Hortvet (^oH). Samples were diluted with three parts of water to one part of mix (Ohmes et al., 1998). Degrees Hortvet were converted to degrees Celcius 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 1.1 L (one quart) plastic freezer containers for the yield stress tests (Bes-Pack, Webster Industries, Peabody, MA) and 0.18 L (6 oz) plastic freezer containers (Sweetheart Plastic Food Cups, Sweetheart Cup Company, Inc., Chicago) for compression 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^{o}C) for storage.

Evaluation of Mix and Frozen Ice Cream

Gross composition of the mix (total fat, protein, moisture, total solids) were conducted 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 was obtained 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 and one mL of each diluted sample 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^{o}C for 48 h and psychrotrophic bacteria counts were incubated at 7 ^{o}C for 10 days on aerobic count Petrifilm (Marshall, 1993). Coliform counts were incubated at 32 ^{o}C for 24 h on coliform count Petrifilm (Marshall, 1993).

Textural characteristics of the ice cream were determined by instrumental methods and human perceptual methods. Yield stress was determined by the vane method suggested by Steffe (1992) and described by Briggs et al. (1996). An universal testing machine (Instron Model 1125, Instron, Canton, MA) equipped with a 120 N-m (2000 in-lb) torsion load cell was used to measure the torque required to rotate a vane through ice cream samples. The torsion cell was calibrated electronically by the Instron. The initial vane size was the same as that reported by Brigg's et al. (1996); however, preliminary testing showed that there was more sensitivity on the Instron with the 120 N-m load cell using a larger vane. Two larger vanes were used to determine if yield stress was influenced by vane size. The height of the vanes were both 5.1 cm. The width of the base of the smaller vane was 3.6 cm and the width of the base of the larger vane was 4.4 cm. The thickness of the metal on each individual blade was 0.01 cm. The dimensions of

the vane complied with Steffe's (1992) suggested 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 that is submerged in the sample, and Z_2 is the distance from the bottom of the vane to the bottom of the sample. Vane size did have an effect on yield stress values of vanilla ice cream. The smaller vane produced a yield stress value of 4.7 kPa whereas the larger vane produced a value of 7.5 kPa. The larger vane was used for the rest of the analyses.

For each sample, the vane was cooled in ice water prior to testing. While testing, the vane was held by a chuck to prevent rotation of the vane. The 1.1 L ice cream sample was held in place by a metal shell with dimensions of 9.2 cm by 9.2 cm. The sample was raised up into the vane so that the vane plus 1.3 cm of the shaft was submerged in the ice cream. To determine if yield stress was affected by rotational speeds, samples hydrolyzed with the fungal-derived lactase were tested at various speeds. These speeds were: 0.01 rpm, 0.1 rpm, 1 rpm, and 5 rpm. Maximum torque readings were obtained on each ice cream. Temperatures of the ice cream (HH64 Thermometer, Omega Engineering, Inc., Stamford, CT) were taken following each test.

The samples hydrolyzed with the yeast lactase were used to determine if the vane method could be used to determine a difference in shear stress due to lactose hydrolysis. The specimen rotational speed was set at 0.1 rpm and the x-y recorder was set for 12.7 cm per min (5.0 in/min). Maximum torque readings were obtained on each ice cream. Temperatures of the ice cream (HH64 Thermometer, Omega Engineering, Inc., Stamford, CT)) were taken following each test. The results of both tests were reported as yield stress and recorded in pascals by converting maximum torque to yield stress using the following equation:

$$\tau_y = T_m / \{\pi D^3/2 (H/D + 1/ 3)\}$$

where τ_y is the material yield stress, T_m is the maximum torque, and D and H are the vane dimensions (Dzuy and Boger, 1983).

Firmness of the frozen ice cream hydrolyzed by the yeast-derived lactose was determined following the analytical method described by Lindamood et al. (1989). Samples from all replications were evaluated the same day to ensure similar test conditions. These analyses were conducted using a universal testing machine (Instron Model 100, Instron, Canton, MA). A cylindrical probe (6.4 mm), precooled in ice water, was mounted on a 5 kg transducer and used to take compression measurements of ice cream samples. Temperature readings of each ice cream were made following each test. Cross-head speed was set at 20 mm/min for a depression of 10 mm. The firmness was measured as energy, and converted to Joules by multiplying energy (gm-cm) by 9.80665×10^{-5} (Weast and Selby, 1967).

Human subjects were used to determine the ease of dippability for each yeast-derived lactose hydrolysis treatment. At least sixteen panelists for each testing period (three testing periods) were recruited from the Food Science and Technology Department and shown the proper way to dip ice cream (Marshall and Arbuckle, 1996). A multiple paired comparison test was used to evaluate ease of dippability among samples. The panelists were presented with one pair of samples at a time (0% and 80%, 0% and 100%, or 80% and 100%) in a random order. Each panelist evaluated three pairs of samples and identified which sample was easier to dip within each pair. The panelists dipped the ice

cream samples contained in 4.6 L containers from a cooler maintained at -16.5°C. The samples were assigned a three digit code and panelists were instructed to dip samples in an assigned randomized order.

Statistical Analysis

The complete experiment was replicated three times. For the fungal enzyme treatment, one level of hydrolysis (75%) was tested against a control (0% hydrolysis). The yeast enzyme treatment had three levels of hydrolysis (0%, 80%, and 100%) analyzed. At least two observations (duplicate measurement) for each analysis were taken (fat, protein, lactose, sucrose, moisture, total solids, firmness, dippability, yield stress).

Linear regression was completed for each dependent variable using Microsoft Excel '97 software (Microsoft Corporation, Redmond, WA). Minitab (Release #11, Minitab Inc., State College, PA) software was used to conduct analysis of variance on firmness, yield stress, 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.

At least sixteen independent observations (number of panelists) were used for each of the three repetitions of the dippability test. Difference between "ease of dipping" samples was decided at alpha 0.05 and Friedman's analysis was utilized to examine the data (Meilgaard, 1991).

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 15.75 +/- 0.74 mg/ml mix. Percentage moisture and total solids were 65.44% and 34.56%, respectively. All microbial counts were below detectable limits. During freezing, overrun was maintained at 76 +/- 1.66% for all replications.

Hydrolysis

The target level of lactose hydrolysis for the fungal enzyme was 75%. The hydrolysis level achieved was 74 +/- 1% (Table 1). The target hydrolysis levels for the yeast-derived enzyme was 80% and 100%. The actual hydrolysis levels achieved were 82 +/- 2% and 98 +/- 0.5%, respectively (Table 1). These values are much more precise than those achieved in a previous study by Matak (1999). The previous study used the "dosage method" to achieve desired hydrolysis levels, whereas this current study utilized the more accurate "dilution method" of enzyme addition. Sucrose hydrolysis was assessed to determine the specificity of the lactase enzyme (Table 1). Because there was no significant hydrolysis of sucrose, the enzymes were considered to be lactose specific.

During preliminary testing, it was determined that the addition of 2.5 mL yeast enzyme and 13 mL fungal enzyme to 2.3 L mix for 24 h at 4^{o}C was required to accomplish 100% lactose hydrolysis. The amount of fungal enzyme required was much greater than that of the yeast-derived enzyme. The ice cream treated with the fungal lactase enzyme was discovered to have an off-flavor. This off-flavor was due to the high levels of enzyme in the mix. For this reason, textural characteristics of the ice cream treated with the fungal lactase were examined and no sensory tests were performed.

Freezing point of the control ice cream mix was -1.66^{o}C. The ice cream treated with 75% fungal-based enzyme had a freezing point of -1.76 ^{o}C. This value is higher than the freezing points of the ice cream samples treated with 80% and 100% yeast-based enzyme (–1.72 ^{o}C and –1.75 ^{o}C, respectively). It is possible that the fungal enzyme may have hydrolyzed a carbohydrate other than lactose or sucrose. The freezing points of the treatment groups were determined to be significantly lower than the freezing point of the control ice cream as determined by Tukey's test ($p < 0.05$). The freezing points were very similar to freezing points reported in the previous study (Matak, 1999) when the mix was hydrolyzed with various levels of enzyme to achieve a targeted level hydrolysis. Lindamood et al. (1989) calculated freezing points by the methods of Iversen (1983). 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 had a calculated freezing point of -1.45 ^{o}C. Lindamood et al. (1989) also determined that ice cream mixes with 25%, 50%, and 100% lactose hydrolysis had calculated freezing points of -1.62 ^{o}C, -1.67 ^{o}C, and -1.92 ^{o}C, respectively. A wider variation in freezing point values may have existed because Lindamood et al. (1989) had a contaminated sample. Differences may have also been due to variations in sugar compositions of the mixes.

Textural Studies

The vane method, a technique described by Steffe (1992) to determine yield stress of foods, was modified and used to compare temperature and level of lactose hydrolysis with the torque required to rotate a four-bladed vane through ice cream samples. Changing the torsional speed of the Instron resulted in differences in yield stress of vanilla ice cream (0% hydrolysis)(Figure 1). Although the speed of 1 rpm obtained the best R^2 value (0.95) for the correlation of yield stress versus temperature, it was decided to use the speed of 0.1 rpm ($R^2 = 0.80$) because the apparent slope difference between 1 and 5 rpm versus 0.1 and 0.01 rpm may have been the result of a dynamic effect. The results from the slower speeds were consistent with that of a static test. This investigation also demonstrated that as the rotational speed increased, the yield stress also increased. Dzuy and Boger (1983) compared the effect of rotational speed on the measured yield stress of bauxite residue suspensions (red mud). It was determined that at high rotational speeds ($x > 8$ rpm), significant viscous resistance together with instrument inertia and insufficient damping may have introduced errors to the measured maximum torque and hence to the calculated yield stress. From their 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 yield stresses of 0% and 75% lactose hydrolyzed ice cream (fungal lactase source) at 0.1 rpm cross-head speed are shown in Figure 2. This graph clearly shows that regardless of temperature, the 0% lactose hydrolyzed ice cream had much higher yield stresses ($R^2 = 0.80$) than that of the 75% lactose hydrolyzed samples ($R^2 = 0.75$). This is significant in showing that the 75% sample has a lower yield stress, requiring less force to cause flow, which may contribute to "ease of dippability". Briggs et al. (1996) suggested that the ability of ice cream to be dipped is a direct consequence of yield stress. The vane method may offer an objective way to evaluate the flow behavior of frozen desserts (Briggs et al., 1996).

Yield stress for ice cream hydrolyzed with yeast lactase, as a function of temperature, is displayed in Figure 3. There was no significant difference between the 80% and the 100% lactose hydrolyzed samples. The 100% hydrolyzed sample had a very low R^2 value (0.34). This implies that, for this sample, temperature did not influence yield stress. Briggs et al. (1996) reported that, at lower temperatures, a difference in yield stress was less noticeable. Briggs et al. (1996) could find no acceptable curve for yield stress as a function of temperature for a vanilla or chocolate-flavored ice cream. Their results did not produce consistent trends and it was hypothesized that yield stress was affected by the amount of total solids in the mix, product composition, and overrun (Briggs et al., 1996).

Compression measurements were taken on a universal testing machine (Figure 4). Firmness was measured by work (joules) required to penetrate with a pre-cooled cylindrical probe an ice cream sample. Temperature readings were taken during each measurement. The effect of lactose hydrolysis (yeast-based lactase enzyme) on the textural firmness of ice cream are displayed in Figure 4. The values shown on the graph represent measurements taken in the temperature range of $-17.9\,^{o}C$ and $-16.9\,^{o}C$. It was determined that there was a relationship between the firmness of lactose hydrolyzed ice cream (0%, 80%, and 100%) and temperature ($R^2 = 0.98$, 0.99, and 0.97, respectively).

The treatment samples are significantly different from the control, but not different from each other.

The R^2 values from the compression test are significantly higher than from that of the vane test. This may be due to the increased sensitivity of the transducer (5 kg) used for the compression measurements versus the 120 N-m (2000 in-lb) torsion load cell used for the vane measurements. Matak (1999) offered that differences in melting rates may be caused by differences in initial sample sizes. The core of the smaller sample size may reach a higher temperature faster than that of the larger sample, resulting in faster melting (Matak, 1999). The sample size of the ice cream used to take compression measurements (0.18 L) was smaller than the samples used for the vane measurements (1.1 L). The recorded core temperature of the smaller sample may have been more representative of the entire sample than the recorded core temperature of the larger sample. This would result in more accurate temperatures of the samples at the time of testing.

Lindamood et al. (1989) suggested that ice cream softness and extrudability can be enhanced by freezing point depression. Matak (1999) reported that samples treated with high levels of lactase were consistently ranked "easier to dip" than the control sample which was not treated with the lactase enzyme. In the present study, a group of 16 untrained panelists determined that there was a statistical difference in "ease of dippability" between the control (0% hydrolysis) and both the 80% and 100% lactose hydrolysis level. There were no perceived difference between the 80% and 100% treatments. This test was repeated three times.

CONCLUSION

Consumer needs and expectations are pushing the dairy industry to expand product lines to include more specialty items. The enhancement of dipping characteristics at home freezer temperatures is one of the new developments taking place. The hydrolysis of lactose in the ice cream mix is one way to create a softer, more extrudable product. The results of this study implied that the effect of lactose hydrolysis on the dipping characteristics of ice cream could be evaluated successfully by three different methods: the vane method, compression measurements, and human evaluation. This study also supports the dilution method of enzyme addition over the dosage method as a more accurate process of achieving target hydrolysis levels.

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Table 1. Hydrolysis levels of ice cream mixes with varying levels of lactase enzyme from two different microbial sources: *Aspergillus oryzae* and *Kluyveromyces lactis.*

Target lactose hydrolysis[a] (%)	Percent lactose hydrolysis[b] (mean[d] +/- s.e.)	Percent sucrose hydrolysis[b] (mean[d] +/- s.e.)
75[b]	74 +/- 1	0 +/- 0
80[c]	82 +/- 1.5	1.25 +/- 1.25
100[c]	98 +/- 0.5	0 +/- 0

[a] Ice cream mixes were inoculated with specified amounts of lactase enzyme and stored 24 h at 4°C for hydrolysis.

[b] Lactase enzyme source: *Aspergillus oryzae*.

[c] Lactase enzyme source: *Kluyveromyces lactis*.

[d] Averages based on three replications.

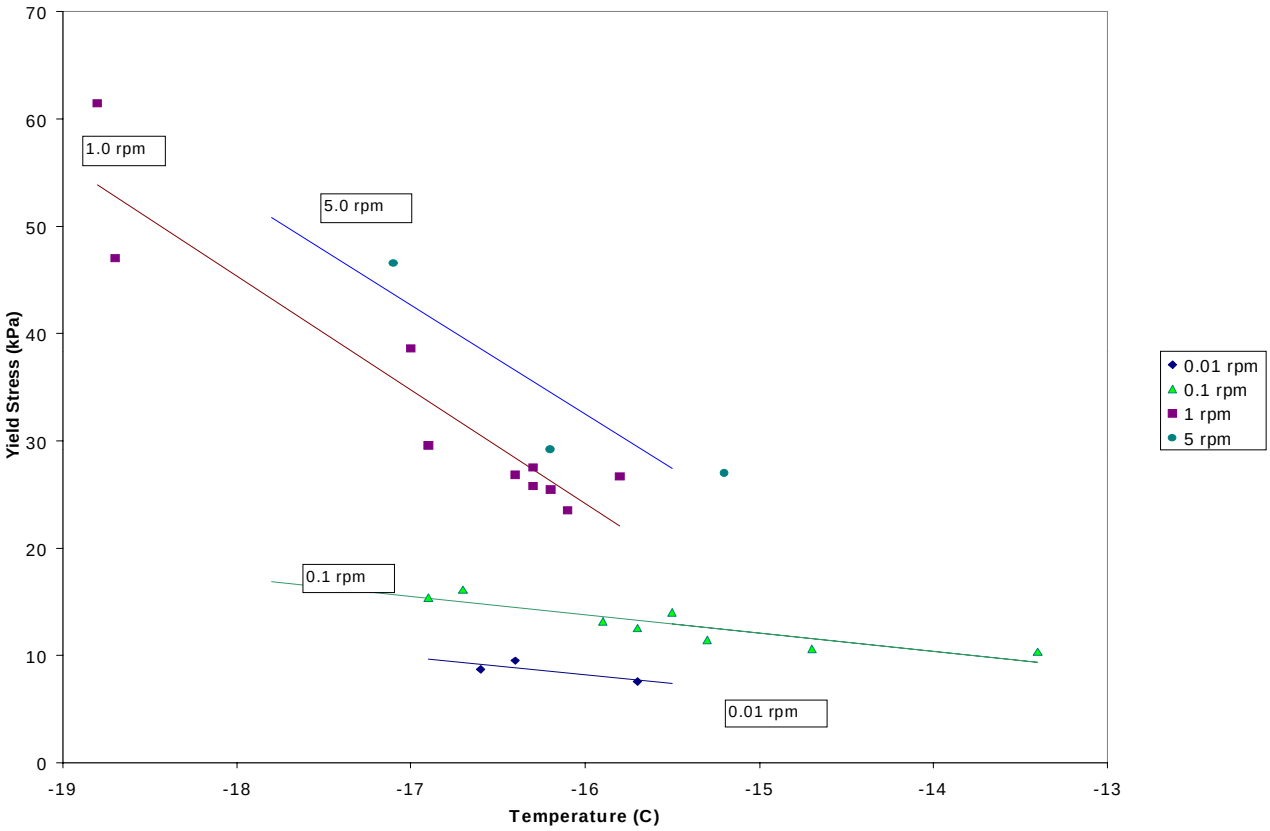

Figure 1. Yield stress as related to temperature of vanilla ice cream at different vane rotation speeds. Measured by the vane method. (5.0 rpm: Y = -129.926 - 10.1546X, R^2 = 0.81; 1.0 rpm: Y = -145.646 - 10.6133X, R^2 = 0.95; 0.1 rpm: Y = -13.516 - 1.70699, R^2 = 0.80; 0.01 rpm: -17.629 - 1.61493, R^2 = 0.62).

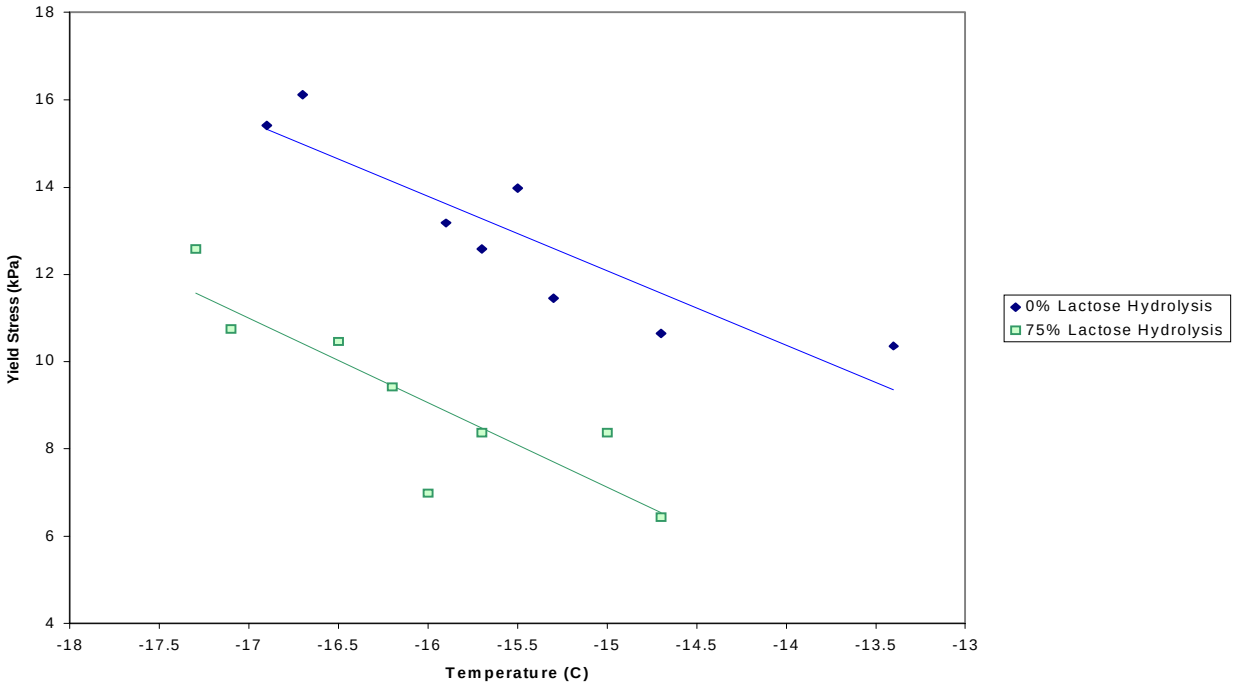

Figure 2. Yield stress as related to temperature of ice creams with 0% ($Y = -13.516 - 1.70699X$, $R^2 = 0.80$) and 75% ($Y = -21.8064 - 1.92865X$, $R^2 = 0.75$) lactose hydrolysis. Hydrolysis was achieved using lactase from the fungal source *Aspergillus oryzae*. Measured by the vane method at 0.1 rpm. The yield stresses of each treatment (0% and 75% lactose hydrolysis) are significantly different ($p < 0.05$) as determined by Tukey's test.

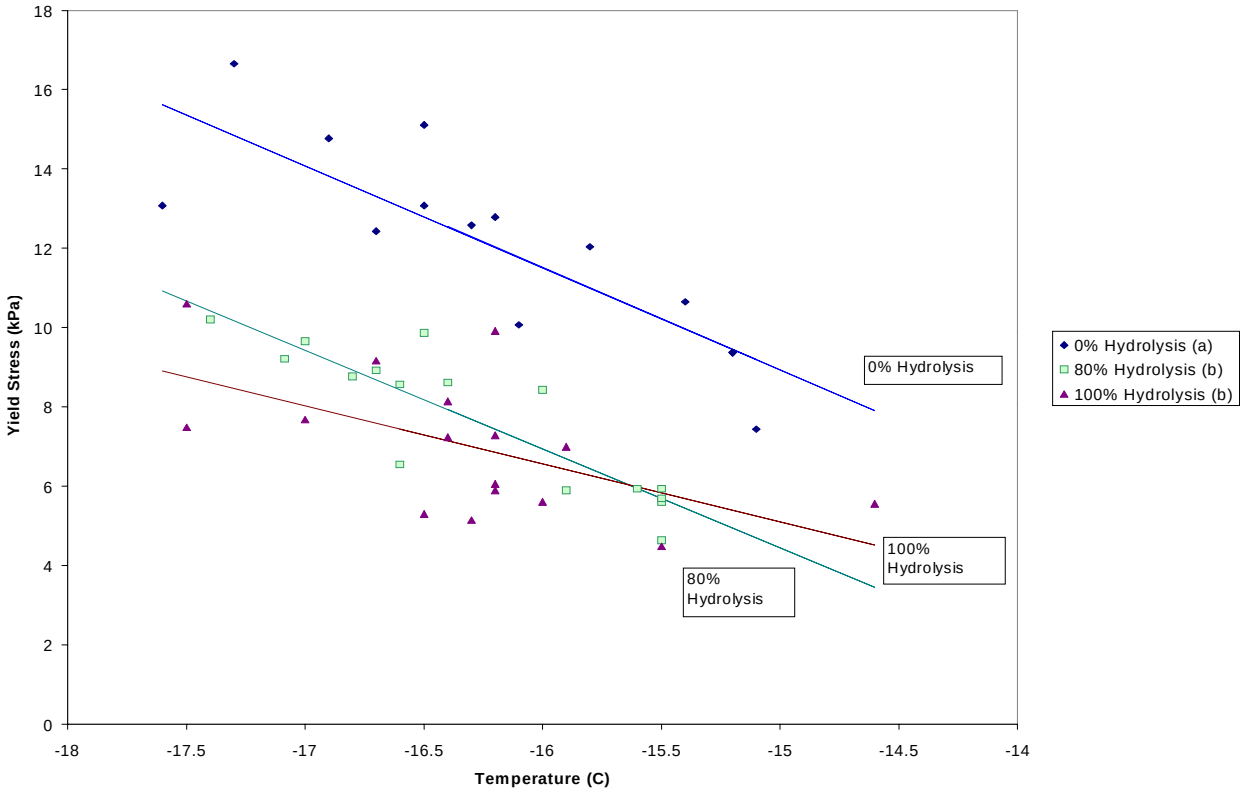

Figure 3. Effect of hydrolysis of lactose on the yield stress of ice creams with 0% (Y = -13.0297 - .26201X, R^2 = 0.70), 80% (Y = -33.0815 - 2.50132X, R^2 = 0.78) and 100% (Y = 016.8101- 1.46098X, R^2 = 0.34) lactose hydrolysis. Hydrolysis was achieved using lactase from the yeast source *Kluyveromyces lactis*. Measured by the vane method at 0.1 rpm.

[a, b] Values designated with different letters are significantly different ($p < 0.05$) as determined by Tukey's test.

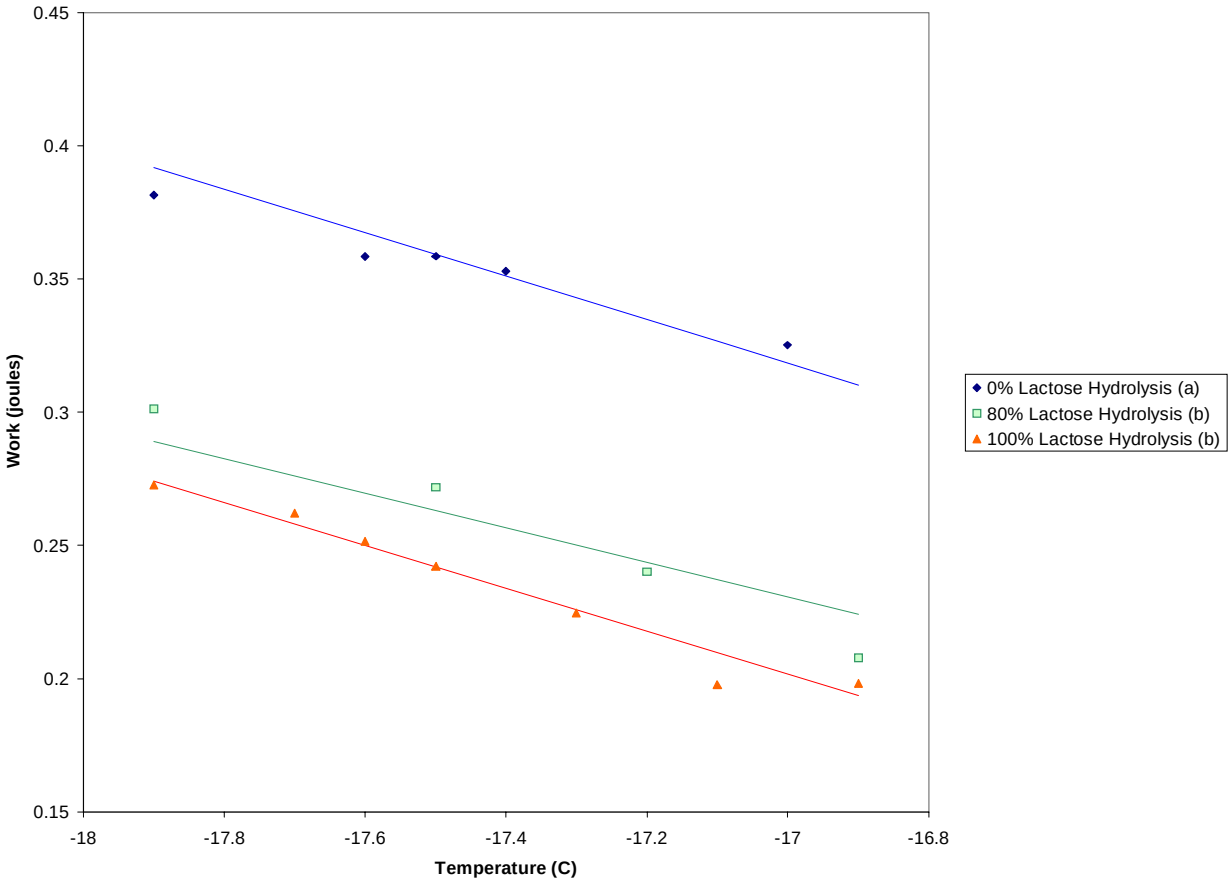

Figure 4. Effects of hydrolysis on the textural firmness of ice cream as shown by Compression measurements. Hydrolysis was accomplished using enzyme from the yeast source *Klyvermyces lactis*. The relationship between firmness of lactose hydrolyzed ice cream is related to temperature (0%: Y = -0.71274 – 0.06112X, R^2 = 0.98; 80%: Y = -1.39254 – 0.09489X, R^2 = 0.99; 100%: Y = -1.16816 – 0.08058X, R^2 = 0.97). The temperature range of interest (-16.9°C to -17.9°C) is shown on this graph.

[a, b] Values designated with different letters are significantly different ($p < 0.05$) as determined by Tukey's test.

APPENDIX A

Sample Preparation for Lactose Assay:

1. Weigh out 2 grams of liquid mix (or melted ice cream). Add 20 mL of 12% (w/v) tricloroacetic acid, mix and filter (Whatman's #1 filter paper).
2. Adjust the pH of 10 mL of the clear supernatant to 7.0 with 1N NaOH and bring the sample up to 25 mL volume with dH2O.
3. Follow the directions in the Boehringer-Mannheim Test Kit (Lactose/D-Galactose test kit, Boehringer Mannheim, Indianapolis).

APPENDIX B

Sample Preparation for Sucrose Assay:

1. Weigh out 1 g sample into a 100 mL volumetric flask. Add approximately 60 mL water and incubate for 15 min at 70^{o}C. Agitate samples periodically.
2. Add 5 mL Carrez-I-solution (3.60 g potassium hexacyanoferrate-II X 3 H2O/100 mL, 5 mL Carrez-II-solution (7.20 g zinc sulfate X 7 H2O/100ml), and 10 mL NaOH (0.1 mol/L) for clarification. Shake rapidly after each addition. Allow sample to adjust to room temperature and fill up to 100 mL mark on flask. Filter (Whatman's #1 filter paper).
3. Use the clear, possibly opalescent solution diluted according to the dilution table for the assay.
4. Follow the directions in the Boehringer-Mannheim Test Kit (Sucrose/D-Glucose test kit, Boehringer Mannheim, Indianapolis).

APPENDIX C

SAMPLE: Preparation and Methods for Protein Assay

Materials:
Ice cream mix (10% milkfat)
Concentrated Dye Reagent (from Bio-Rad Protein Assay)
Protein Standard (Bovine Serum Albumin)(from Bio-Rad Protein Assay)

Methods:
1. Dilute dye reagent (1:4 with distilled water)
2. Filter dye through Whatman's #1 filter paper.
3. Use filtrate for assay.
4. Number test tubes for 0, 0.2, 0.4, 0.6, and 0.8 protein standards.
5. Prepare standard curve dilutions according to quantity of protein in the BSA used. The numbers in step 4 correspond to final protein concentrations in units of mg/mL.
6. Place 1 mL ice cream mix in a 10 mL volumetric flask. Fill the flask with distilled water to the fill line. This is a 1:10 dilution.
7. Mix 0.5 mL diluted sample with 4.5 mL distilled water to give 1:100 dilution.
8. Add 0.1 mL diluted sample and standard curve solutions into labeled test tubes.
9. Add 5 mL filtered dye reagent to each tube and vortex.
10. Incubate for at least 5 min but no longer than 1 hr at room temperature (absorbance will increase over time)
11. Measure absorbance at 595 nm.

APPENDIX D

Sample Human Subjects Form

Virginia Tech

Human Subjects Forms for Sensory Evaluation

Virginia Polytechnic Institute and State University
Informed Consent for Participation in Sensory Evaluation

Title of Project: Lactose Hydrolysis by Fungal and Yeast Lactase: Influence on Freezing Point and Dipping Characteristics of Ice Cream

Principal Investigator: Kristen Matak

I. THE PURPOSE OF THIS PROJECT

You are invited to participate on a sensory evaluation panel about ice cream. The purpose of this panel is to determine if there is a detectable difference in the sweetness and coldness of different ice cream samples. The panel will also be used to determine if there is a detectable difference in the ease of dipping the ice cream samples.

II. PROCEDURES

There will be __12__ sessions over a period of __3__ months involving about __5__ minutes at each session. You will be presented with approximately __4__ samples at each session. As a panelist, it is critical to the project that you attend each session. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to other samples.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, list them in the following space.

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide the following information that may be helpful: to determine if lowering the freezing point of ice cream samples yields a softer, sweeter product. You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have an unknown food allergy.

IV. EXTENT OF ANONYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

VI. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

VIII. SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study which will require: (list sessions to be attended or other requirements.)

_____

Signature/Date

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes.

Address _____

Phone _____

----------(tear off)----------

IX. SUBJECT'S PERMISSION (provide tear off for human subject to keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study which will require: (list sessions to be attended or other requirements.)

_____

Signature

Should I have any questions about this research or its conduct, I should contact:

Kristen Matak (540) 231-3037
Investigator/Phone

Dr. Susan Duncan (540) 231- 8675
Faculty/Phone

Tom Hurd (540) 231-5281 (540)231-6077
Chair, IRB/Phone for Research Division

APPENDIX E

Sample Scorecard for Sensory Evaluation

RANKING TEST FOR SWEETNESS AND COLDNESS

Sample Code: _____ **Judge Number: _____**

Product: Ice Cream Samples

Taste each sample and rank them in order of increasing coldness. You may rinse your mouth with water between each sample.

Code#	Code #	Code#	Code#
Least Cold			Most Cold

Taste each sample and rank them in order of increasing sweetness. You may rinse your mouth with water between each sample.

Code#	Code #	Code#	Code#
Least Cold			Most Cold

Please answer the following question:

1. What is your age?
   a. _____ 18-24
   b. _____ 25-30
   c. _____ 31-45
   d. _____ 46-64
   e. _____ 65 and up

2. How often do you eat ice cream?
   a. _____ > once a week
   b. _____ once a week
   c. _____ once a month
   d. _____ a few times a year
   e. _____ hardly ever

Sample Scorecard for Sensory Evaluation

EASE OF DIPPING
SCORECARD

Sample Code: _____ **Judge Number: _____**

Product: Ice Cream Samples

Dip approximately one scoop of ice cream from each container. Evaluate each sample for ease of dippability and rank them in order of increasing hardness.

Code#	Code#	Code#	Code#
Easiest to Dip			Hardest to Dip

THANK YOU FOR COMPLETING THE TEST!

Sample Scorecard for Sensory Evaluation

EASE OF DIPPING
SCORECARD

Sample Code: _____ **Judge Number: _____**

Product: Ice Cream Samples

Dip approximate one scoop of ice cream from each container and indicate which sample is easiest to dip by circling the appropriate number code.

Dipping Order:

1. _____ Code #
2. _____ Code#

Dipping Order:

1. _____ Code#
2. _____ Code#

Dipping Order:

1. _____ Code#
2. _____ Code#

THANK YOU FOR COMPLETING THE TEST!

APPENDIX F

Study One: Raw Data

	Target Lactose Hydrolysis (%)	Actual Hydrolysis (%)	Freezing Point (C)	Work (joules)	Melting Point (mL/min)
Trial One	0	0	-1.629	0.3906	1.48
	25	29	-1.656	0.2427	1.55
	50	38	-1.665	0.1480	1.55
	75	61	-1.700	0.1145	1.56
Trial Two	0	0	-1.641	0.2895	1.36
	25	20	-1.644	0.1669	1.41
	50	32	-1.685	0.1170	1.58
	75	83	-1.739	0.0879	1.69
Trial Three	0	0	-1.630	0.3630	1.44
	25	35	-1.616	0.2811	1.55
	50	61	-1.644	0.1526	1.67
	75	70	-1.733	0.1038	1.67

Study One: Sensory Dipping Panel Data

Target Hydrolysis (%)	0	25	50	75
Rank Sum[1]	160	123	111	86
T-Statistic		35.58		
LSD rank		24.79		

1 The sum of forty-eight responses when asked to rank the four hydrolysis levels on the basis of ease of dippability (4 = hardest to dip and 1 = easiest to dip).

VITA

Kristen Erica Matak was born on May 23, 1974 in Jersey City, New Jersey. She graduated from Vernon Township High School in Vernon, New Jersey in 1992. During the fall of 1992, she began studies at West Virginia University in Morgantown, West Virginia, and graduated in May of 1996 with a B.S. in Human Nutrition. In the fall of 1997, she began work towards a Master of Science degree in Food Science at Virginia Polytechnic Institute and State University.