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Preparation of hydrolyzed lactose syrup from cottage cheese whey

Magdy Hefnawy

To the Graduate Council:

I am submitting herewith a dissertation written by Magdy Hefnawy entitled "Preparation of hydrolyzed lactose syrup from cottage cheese whey." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Hugh O. Jaynes, Major Professor

We have read this dissertation and recommend its acceptance:

Ada Marie Campbell, Bobby Joe Demott, Sharon Melton

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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and recommend its acceptance:

B. J. Demott

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PREPARATION OF HYDROLYZED LACTOSE SYRUP FROM COTTAGE CHEESE WHEY

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Magdy Hefnawy

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ABSTRACT

A concentrated syrup with 80% solids was prepared from whey after hydrolysis of lactose. Four enzymes were evaluated for efficiency, and Maxilact 20,000 was used in the study. Appropriate treatments for hydrolysis of lactose, deproteinization, decolorization, demineralization and concentrations were established.

The syrup had a pleasantly sweet flavor, was light yellow in color, and had an apparent viscosity of 2050 centiposes. When corn syrup (3.75% by wt) in vanilla ice cream was replaced by the hydrolyzed lactose syrup, there was no reduction of quality as judged by an expert panel.

CHAPTER I

INTRODUCTION

In the United States, over one billion pounds of cottage and creamed cheeses were manufactured during 1977 (Anonymous, 1978a). For each pound of cottage cheese, approximately 6 pounds of a "yellowish-green fluid" known as whey are produced. This fluid contains roughly half the solids of the whole milk from which it is derived. These solids consist of approximately 70% lactose, 12% protein, 9% minerals plus small amounts of fat and lactic acid. Only 53.2% of the total whey produced in 1977 was converted to marketable products (Anonymous, 1978b). One reason for the large amounts of unprocessed whey solids is the unfavorable economic involved in assembling and processing whey from the smaller cheese factories (Braatz, 1974). The processing and marketing of the large quantity of cottage cheese whey that is now being disposed of by means other than processing is a challenging task. More strict environmental regulations and the increasing load on many existing municipal sewage treatment plants are creating a serious problem in the cottage cheese industry. Many cottage cheese manufacturers, because of their size, are in a disadvantageous position in whey disposal. Their volume is too small to justify the installation of disposal or processing equipment, and at the same time municipal sewage plants are refusing to treat their whey because of maximum loading on their plants.

These circumstances have led cottage cheese manufacturers to seek methods of converting their whey into saleable products.

One approach to solve this problem is the hydrolysis of the lactose in whey to glucose and galactose, concentrating it to a syrup and using it as a sweetner in dairy products. As the cost of sweetners from cane and corn continues its dramatic climb, certain segments of the food industry are looking for alternative sources of sugars (Pitcher, 1974). The availability of purified lactase enzyme preparations on a commercial scale made the lactose hydrolysis an attractive commercial process.

This study dealt with the evaluation of some lactose enzymes commercially produced in the United States, the production of concentrated hydrolyzed lactose syrup and evaluation of ice cream in which the hydrolyzed lactose syrup was used as an ingredient in the mix. The main objective of the study was converting cottage cheese whey into a product which small dairy plants can use as an ingredient in ice cream mix replacing corn syrup.

CHAPTER II

REVIEW OF LITERATURE

A. LACTOSE HYDROLYSIS

Lactose hydrolysis has been studied for many years. Lactose may be hydrolyzed by dilute solutions of strong acids (Ramsdell and Webb, 1945; Ney and Wirotama, 1970; Coughlin and Nickerson, 1975; Hagget, 1976; Vujicie et al., 1977; Demaimay, 1977), and also by the enzyme lactase (Knopfmacher and Salle, 1941; Wallenfels et al., 1960a,b; Wendorff et al., 1971; Kisza et al., 1973; Kosikowski and Wierzbicki, 1973; Sukegawa, 1974; Edelsten, 1974; Guy et al., 1974). The hydrolysis of lactose by acid was first reported in 1812, and its hydrolysis by a lactase preparation in 1883 (Whitter, 1926). The hydrolysis of lactose into glucose and galactose in a variety of milk products has been investigated to solve the problem of lactose intolerance (Paige et al., 1971) as well as some dairy production problems (Talley and Hunter, 1952; Tumerman et al., 1954; Thompson and Brower, 1974; Thompson and Gyuricsek, 1974). Several advantages are claimed as a result of the hydrolysis of lactose in dairy products such as increasing sweetness, changing crystallization patterns, lowering the freezing point and varying viscosity and humectant properties (Bauvy, 1974; Nickerson, 1974; Woychik and Holsinger, 1977; Guy and Edmondson, 1978).

Acid Hydrolysis

Lactose is quite resistant to acid hydrolysis. Organic acids such as citric that easily hydrolyze sucrose are unable to hydrolyze lactose under the same conditions (Webb et al., 1974). Ramsdell and Webb (1945) studied the acid hydrolysis of lactose to prepare a hydrolyzed lactose syrup, and reported a destructive effect on the proteins of dairy products, milk or whey. Ney and Wirotama (1970) claimed that acid hydrolysis of lactose occurred more rapidly in whey than in pure lactose solution, considerable hydrolysis occurring at 90°C and pH 4.7. It was suggested that whey protein may exercise a catalytic effect on lactose hydrolysis. Lactose in fresh cottage cheese whey and in aqueous solutions was successfully hydrolyzed in 0.5, 1, 2 and 3 N HCl at 50, 60 and 70°C (Coughlin and Nickerson, 1975). The hydrolysis rates in both whey and aqueous lactose solutions were comparable at 50 and 60°C, whereas at 70°C in whey the rate appeared to be 10-20% lower than in aqueous lactose solutions. This was attributed to loss of free glucose through the browning reaction. Lin and Nickerson (1977) compared the acid hydrolysis of lactose in whey and in aqueous solution. Acid hydrolysis of concentrated whey with sulfuric acid produced a more severe browning reaction and off flavor than the hydrolysis of lactose in aqueous solution. Vujicie et al. (1977) found that lactose hydrolysis by sulfuric acid was noticeably slower than by hydrochloric acid. A cation exchange resin (Amberlite IR 118-4) was used successfully to

catalyze the hydrolysis of lactose to glucose and galactose at 90°C in pure solutions and in demineralized permeate obtained from ultrafiltration of whey (Haggett, 1976). Demaimay (1977) studied the hydrolysis of lactose in pure lactose solution and in deproteinized, demineralized whey using cation exchange resin and Maxilact lactase enzyme for 24 hr at 20°C. Although both treatments gave about 75% lactose hydrolysis, the cation resin catalytic hydrolysis method produced a hydrolyzed syrup with better quality (color and odor) from the lactose solution and the whey. Gillies (1974) stated that acid hydrolysis was not practical because exceptionally severe conditions are required.

Enzymatic Hydrolysis

Lactase, or β-D galactoside - galactohydrolase (Bauvy, 1974), is not only active on its natural substrate lactose but also on other substrates which contain a β-D-galactosidic bond. β-D-galactosidic activity is widely distributed in nature, having been found in many tissues of higher animals, in yeasts, in molds, in bacteria, in birds and in plants (Webb et al., 1974). β-galactosidase occurs in the emulsions of some Rosacea, in Kefir grains, almonds, tips of wild roses and seeds of soybeans, alfalfa and coffee (Wehmer and Hadders, 1933; Sumner and Somers, 1953; Stenlid, 1957; Wallenfels and Malhotra, 1960; Woychik and Holsinger, 1977). The enzyme has been found to be present in the fungi Aspergillus oryzae, Aspergillus niger, Aspergillus flavus (Neuberg and Rosenthal, 1924; Guagnini and Jacovkis, 1942;

Estienne et al., 1947; Wallenfels and Malhotra, 1960) and in Neurospora (landman and Bonner, 1952; Landman, 1954; Zalokar, 1959). Although β-galactosidase occurs in a number of yeast species, the enzymes from Saccharomyces fragilis and Saccharomyces lactis have been studied most widley (Gilmour, 1957; Sukegawa, 1974). The enzyme has been obtained from various strains of Lactobacillus and Escherichia coli (Wallenfels and Malhotra, 1960), and is known to occur in a number of strains of the genus Shigella (Rickenberg, 1960; Sarkar and Luria, 1962), in Pneumococci (Fleming and Neill, 1927), Staphylococci (Creaser, 1955; Hancock, 1958), and Corynebacteria (Bermerts and DeLey, 1957). Among the animals, the enzyme is found in nails and the intestines of dogs, rabbits, calves, sheep, goats, rats, rams, hulls, and boars (Cajori, 1934, 1935; Conchie et al., 1959a,b; Wallenfels and Malhotra, 1960).

β-galactosidases isolated from different sources have different properties. Marise et al. (1973) studied the characteristics of β-galactosidase from yeast and E. coli. The enzyme from E. coli. was stable up to 45°C while the yeast enzyme rapidly lost its activity above 30°C. The optimum pH was 6.6 to 7.5 for bacterial enzyme and 6.0 to 7.0 for yeast enzymes. β-galactosidase derived from Aspergillus niger showed maximum activity at temperatures of 37 to 55°C and pH 3.8 to 4.6 (Wallerstein Co., 1974).

Wierzbicki and Kosikowiski (1973a) studied the kinetics of lactose hydrolysis in acid whey by β-galactosidase from Aspergillus niger. The maximum rate of hydrolysis was at a concentration of 21%

lactose in concentrated whey. Hydrolysis of 75 to 90% of lactose was obtained in 4 - 5 hours at 55°C in whey containing 4% lactose using 40 mg of enzyme. Pasteurized and raw milks were treated with lactase enzyme from Saccharomyces lactis. An enzyme concentration of 25 mg/liter for 48 hours at 4°C hydrolyzed 80% of the lactose in pasteurized milk and 75% in raw milk. An enzyme concentration of 100 mg/liter in pasteurized milk at the conditions hydrolyzed 95% of the lactose and in raw milk 90% (Kosikowski and Wierzbicki, 1973). They reported that color, pH, sedimentation and flavor quality were not adversely affected in milks treated with Saccharomyces lactis lactase (Maxilact 40,000). They found that 1 g Maxilact hydrolyzes 266 g lactose/hour in 5% lactose solution at pH 7 and 22°C. They reported also that the enzyme was stable in milk for weeks or months at 24°C, under sterile conditions, without decrease in activity. In whey it was stable for an equally long time at 4°C, although it was less stable at 24°C. Sukegawa (1974) used purified lactase from Saccharomyces lactis (35,000 ONPG U/g) in raw milk and reported no proteolytic activity toward milk protein, no appreciable effect on flavor quality of the milk, but intensity of sweetness was proportional to lactose concentration.

Bauvy (1975) reported that milk or whey to be treated with lactase should be of good microbiological quality, otherwise lactase activity is inhibited to some extent. He stated that the optimum temperature for hydrolysis, in milk or whey with Saccharomyces lactis, is 30 to 35°C and the pH of whey should be adjusted to at least 6.5 with potassium or ammonium hydroxide.

Lactase from Saccharomyces fragilis was active at pH 6 - 7 but inactive at pH 5 (Wendorff and Amundson, 1971; Popova et al., 1974). The latter observed that the optimum temperature for the enzyme in milk was 30 - 40°C while in concentrated milk at 32 - 44% total solids, it was 40 - 50°C. Wendorff et al. (1971) examined the β-galactosidase activity of Saccharomyces fragilis toward the lactose contained in a variety of milk products concluded the greatest activity occurred in normal whey and reconstituted dried whey. Kisza et al. (1973) stated that the most favorable conditions for hydrolysis by Saccharomyces fragilis lactase with no undesirable side effects were found to be at 8°C for 15 hours. Edelsten (1974) reported that incubation of whole or skim milk with lactase from Saccharomyces fragilis at 31°C for 17 - 19 hours hydrolyzed 98% of the lactose.

Jasewicz and Wasserman (1961) reported that sodium ions and potassium ions strongly activate yeast lactase. It has also been mentioned that the same effect occurred with Escherichia coli lactase (Pomeranz, 1964). Pauvy (1975) and Sahlqvist et al. (1977) reported that the Saccharomyces lactis in milk has higher activity in the presence of potassium ions than sodium ions. Manganese ions offered some protection and serve as a co-factor for the enzyme (Wendorff and Amundson, 1971). Bauvy (1975) reported that low concentrations of manganese are essential in maintaining the enzymes in active structure. Magnesium and cobalt partly replaced manganese in this function. Reithel and Kim (1960) found that the magnesium ion is required for the catalytic activity of β-galactosidase from E. coli.

Heavy metals strongly inhibit β-galactosidase activity (Dahlgvist et al., 1977; Bauvy, 1975; Pomeranz, 1964). Wallenfels and Fischer (1960) interpreted the inactivation effect of heavy metals as due to a nonspecific binding of metal ions that results in changes in the tertiary structure of the enzyme protein.

Immobilized β-Galactosidase

Although lactose hydrolysis can be achieved most simply by the addition of soluble enzyme to milk or whey, immobilized enzyme technology has been evaluated with lactases in an effort to improve the economics of lactose hydrolysis (Pitcher, 1975; Woychik and Wondolowski, 1973; Pastore et al., 1974; Richardson and Olson, 1974; Wierzbicki et al., 1974). Studies of immobilized β-galactosidases from E. coli (Sharp et al., 1969), Saccharomyces lactis (Dahlqvist et al., 1973; Woychick et al., 1974) and Aspergillus niger (Woychick and Wondolowski, 1972, 1973; Olson and Stanley, 1973) have been reported in the literature.

Pitcher (1974) investigated in the feasibility of using immobilized lactase for the commercial hydrolysis of the lactose in cheese whey. Lactase LP, a β-galactosidase from Aspergillus niger, was immobilized on porous silica bodies. The half life of the immobilized enzyme was found to be affected by the purity of whey. He found that NaCl and protein content decreased the enzyme's life. Edwards (1972) and Wierzbicki et al. (1973) reported that a lactase from Aspergillus niger had greater initial activity and stability than lactases from other sources when immobilized on porous glass.

β-galactosidase of Saccharomyces lactis was immobilized on porous glass beads and on, a new support, comminuted collagen (Woychik et al., 1974). The collagen was demonstrated to be comparable to other supports, and because of its hydrophilicity, it may facilitate substrate diffusibility in aqueous systems. The authors reported that enzymatic properties of Saccharomyces lactic β-galactosidase remained essentially unaltered after coupling to either glass or collagen.

Woychik and Holsinger (1977) stated that problems associated with protein adsorption to a variety of enzyme support systems and maintaining acceptable column sanitation levels when working with nutritive substrates, such as milk or sweet whey, further limit its usefulness.

Formation of Oligosaccharides During Enzymatic Reaction

The glycosidases, such as β-galactosidase, are known to catalyze transfer-reactions in which another sugar molecule becomes the acceptor of the β-D-galactase moiety instead of water (Pomeranz, 1964; Bauvy, 1975). This transfer principle is responsible for the formation of oligosaccharides during and after lactose hydrolysis.

Wallenfels (1951) found that lactose hydrolysis by a crude or highly purified lactase preparation from a culture of Aspergillus oryzae resulted in the production, besides glucose and galactose, of three substances which had lower R_f values on paper chromatograms and were less reducing than lactose. Aronson (1952) reported the presence of four

oligosaccharides formed during the hydrolysis of lactose by Saccharomyces fragilis lactase and at least three oligosaccharides formed during hydrolysis of lactose by E. coli lactase. Pazur (1954) isolated four oligosaccharides which were produced during the action of an enzyme extract from yeasts, Saccharomyces, Torulopsis and Candida species, on lactose. The results of tracer experiments showed that the compounds were produced by enzymatic transgalactosidation in which the galactosyl units from lactose were transferred to glucose, galactose, lactose or galactose (1 - 6) glucose.

Wierzbicki and Kosikowski (1973b) studied the formation of oligosaccharides by transgalactosidation reactions during hydrolysis of lactose in acid whey by β-galactosidase from Aspergillus niger. This study showed the presence of five oligosaccharides and these appeared at very low levels, estimated to be 1 to 2% of the total lactose. However, at substrate concentration higher than 4% lactose the total oligosaccharides appeared in larger amounts. Aronson (1952) reported that increasing the substrate concentration increased the amount of oligosaccharide. Roberts and Pettinati (1957) investigated the lactose concentration effects in the enzymatic conversion of lactose to oligosaccharides by Saccharomyces fragilis. They concluded that the percentage of lactose converted to oligosaccharides increased as the initial lactose concentration increased. A maximum conversion of 44.6% was obtained when starting lactose concentration was 35% W/V. They reported the formation of eleven oligosaccharides.

B. REMOVAL OF WHEY PROTEIN

When proteins are the proteins remaining after casein has been removed from skim milk. Prior to 1934, it was generally considered that whey protein consisted of two main components, lactoalbumin and lactoglolumin; and each of them was considered to be a single chemical entity until Palmer, (1934) reported their fractionation. Whey proteins constitute approximately 0.6% of whey (Webb et al., 1974). Rose et al., (1970) stated that the major whey proteins (noncasein serum proteins) are: β-lactoglobulins, α-lactalbumin, bovine serum proteins and the milk immuno-globumins. A number of methods have been developed for the removal of proteins from whey. These methods generally involve the denaturation of the whey proteins by heat coagulation. Rodgers and Palmer (1966) reported that the best precipitation conditions resulted from carrying out the heating process in several stages: (a) preheating the whey at pH 4.6 until coagulation started to take place; (b) heating the whey at about 95^{o}C under conditions of turbulence, and (c) addition of polyelectrolytes. The removal of proteins from whey by precipitation was studied by Roeper (1970). He found that sufficient conditions for precipitation of protein from various types of whey included heating to 80 -85^{o}C for 10 minutes, addition of Ca++ (up to 2000 ppm total) and adjustment of pH with NaOH up to 7.5. Kresheninum et al. (1974) investigated the separation of proteins from cheese whey with precipitation techniques: heating to 90 - 95^{o}C; heating to 90 - 95^{o}C with acidification to pH 3.0 - 3.5 with HCl; heating to 90 - 95^{o}C with

acidification to pH 3.0 - 3.5, holding for 20 minutes and neutralization with $NaHCO_3$ to pH 8.0; and heating to 90 - 95°C with the addition 1% of a 20% $CaCl_2$ solution. They found that the overall protein recovery was most complete (85.1%) for the last technique. However, taking into account other factors, the authors considered that heating to 90 - 95°C with acidification to pH 3.0 - 3.5 was the most expedient for practical use.

Rowland (1937, 1938), Menefee et al. (1941), Harland et al. (1945, 1952), Jenness (1954), Larson and Roller (1955) and Lyster (1970) have demonstrated that the whey proteins were denatured according to the extent of the heat treatment under various conditions. All of these studies were concerned with the denaturation of either the total whey proteins or the globulin and lactalbumin fractions.

Larson and Rolleri (1955) showed that the various proteins in whey were heat denatured at different rates; the immune globulin fraction was denatured first followed by the serum albumin. β-lactoglobulin was affected less rapidly under the same heating conditions while α-lactalbumin was most resistant. Purified β-lactoglobulin and α-lactalbumin as well as cottage cheese whey were investigated for heat denaturation with various additives: NaCl, $CaCl_2$, collagen, sodium hexametaphosphate, $FeCl_3$ and cysteine (Townsend and Gyuricsek, 1974). The authors concluded that neither simple ionic substances nor polyelectrolytes had a substantial general effect, whereas disulfide reduction of some protein components of whey by adding cysteine caused a large increase in the

amount of protein precipitation.

Mularz and Swanson (1953) and Guy et al. (1967) reported that the proteins in cottage cheese whey were more resistant to heat denaturation than similar proteins in milk. They stated that shifting the pH of cottage cheese whey upward prior to heating could be used to increase the rate of protein denaturation. But Lyster (1970) reported that the rate of whey protein denaturation at either 78 or 100°C was independent of pH within the range of 6.2 - 6.9. Outside this range the ratio of denaturation increased.

C. DEMINERALIZATION OF HYDROLYZED LACTOSE SYRUP

Dry whey contains between 7 and 12% ash. Cottage cheese whey contains more ash than wheys resulting from the production of cheddar cheese. This high level of salts limits the use of whey than can be used in many applications.

There are a number of methods which may be used to demineralize whey to increase its suitability for food use. Delaney (1976) reported that the removal processes of minerals from whey fall into two main categories, electromembrane processes and ion exchange resin processes. Electromembrane processes may be divided to four main sub-categories: electrodialysis, transport depletion, electro-osmosis and ion substitution.

Ahlgren (1977) pointed out that electromembrane processes can reduce the ash and titratable acidity content of cottage cheese whey while leaving the protein and lactose content essentially unchanged. He also

stated that the removal of ash constituents by electromembrane processes was not as easy as it was by conventional ion exchange resin processes. Also, electromembrane processes can never give complete demineralization due to effects such as concentration polarization (Delaney, 1976).

Hervé (1974) described the mineralization of whey by ion exchange resins. He reported that the ion exchange process appears to possess numerous advantages which include the possibility of treating any kind of whey (acid or sweet), a much lower investment cost, lower running cost and simpler to use and maintain. He found that after passing through the cation and then the anion exchange resins, the whey was demineralized to the extent of 90 - 99% depending on the quality of the raw whey.

Delbeke (1972) investigated the usefulness of eight cation exchange and eight anion-exchange resins. These resins were proposed by the manufacturers as suitable for whey treatment. Among eight strong acid resins Amberlite IR 120 and Lewatit S 100 gave better results than the other six. During the decationization with Amberlite IR 120 the dry matter loss was 6.1 to 6.8% and the nitrogen loss was 7.2 to 9.4%. In the removal of anions, Amberlite IR A 68 gave the best results of all resins used, and the loss of dry matter amounted to 5.8%, while the loss of nitrogen was 7.4%. The degree of demineralization in this study amounted to practically 90% of the salts present.

Wierzbicki and Kosikowski (1973c) reported that a clear, golden, very sweet syrup without whey taint or saltiness was obtained when

hydrolyzed acid whey was heated to 90°C at pH 4.5 for 5 minutes, cooled to 25°C, and simultaneously treated with sodium hydroxide to pH 9 for precipitation of protein and minerals. The protein, with entrapped precipitated minerals, was removed by centrifugation. The whey was then adjusted to a neutral pH by ion exchange with Dowex 50 or lactic acid and then concentrated at low temperature.

Guy and Edmondson (1978) used Dowex 50 W - X8, 20 - 50 mesh, in the hydrogen ion form to remove cations from hydrolyzed lactose solutions, and Dowex 2-X8, 20 - 50 mesh, in the hydroxyl ion form to remove the anions. In this study the hydrolyzed lactose solutions were decolorized with 1% Norit charcoal and filtered through celite filter-aid.

D. APPLICATION OF HYDROLYZED LACTOSE SYRUP

The idea of the hydrolysis of lactose as an extension of whey utilization is far from new. Several advantages are claimed as a result of the hydrolysis of lactose into glucose and galactose.

Nickerson (1974) stated that hydrolysis of lactose in a variety of milk products can provide at least five advantages: (1) increase in sweetness, which may be an economy with today's high sugar prices; (2) decrease in lactose content, which is important in lactose intolerance in animals as well as in man; (3) change in crystallization pattern, which may result in products with less sandiness or defective texture, or may promote softer confections with delicate texture; (4) lowering of the freezing point, which might be important in ice cream and in

frozen milk; (5) changes in viscosity and humectant properties resulting from hydrolysis.

While the applications for a hydrolyzed lactose in a number of food and feed industries are apparent, its actual use has been very limited. Pomeranz (1964) and Bauvy (1974) noted that the reasons that more work on the application of hydrolyzed lactose has not been carried out may be that commercially feasible lactase preparations have not been available and that the properties of lactases from various sources vary widely.

Bauvy (1975) reported that concentrated whey syrups are extremely sweet, and can be incorporated into ice creams, frozen desserts, bakery products, candies and confections, bread, spreads, and soft drinks. He also indicated that in all these applications, the additional sweetness due to lactose-hydrolysis in the dairy product is realized without the addition of calories. In products where the additional sweetness from lactose hydrolysis is sufficient, there is no need to alter the products natural status by adding a noncaloric sweetner.

Moore (1978) reported that substantial amounts of hydrolyzed lactose could be absorbed by the ice cream, confectionery, baking and canned fruit industries if quality, functional properties, and price were satisfactory. She also reported that a series of four hydrolyzed lactose sweeteners is now commercially available from Aries International Company of Paris, France. These new sugars, Yogaserums, are prepared with enzymatic hydrolysis of lactose to glucose and galactose and conversions of the sugar index from 18 to 72.

Guy and Edmondson (1978) indicated that syrups from hydrolyzed lactose might find application in blending with high solids corn or sugar can syrups or use in high sugar baked goods and in confections.

Wierzbicki and Kosikowski (1973c) suggested using food syrups from acid whey treated with β-galactose Aspergillus niger in processing of swiss-style flavored yogurts, imitation maple syrups, nutrient fruit juices, and puddings. They reported that the choice of hydrolyzed lactose syrup is dependent upon color, flavor, nutrient costs and type of food formulation.

Guy (1978) investigated the partial replacement of nonfat milk solids and cane sugar in ice cream with hydrolyzed sweet whey solids. Two levels of sweet wheys with either 67 or 79% hydrolyzed lactose were used to replace parts of nonfat milk solids and sugar to produce ice cream. The formulations varied in their contents as follows: nonfat milk solids 11 to 5%, sugar 15 to 10% and hydrolyzed sweet whey solids none (control) to 11%, the three ingredients totaling 26%. He found that the initial hedonic flavor and texture scores of ice creams containing up to 5.5% of either of the wheys were not significantly different from those of the controls. He also found that increasing both types of hydrolyzed whey solids above 5.5% significantly decreased mix viscosity and hedonic flavor scores and increased the saltiness of the ice cream.

CHAPTER III

MATERIALS AND METHODS

A. SOURCE OF WHEY AND LACTASE ENZYMES

Cottage cheese whey was obtained from Weigel's Inc., Powell, Tennessee. Whey was stored at $4^{o}C$ until used. Four lactase enzymes were obtained from their respective manufacturers as identified in Table 1.

B. ENZYME EVALUATION

A calculated amount equal to 9,100 ortho-nitrophenyl-beta-D-galactopyranoside (ONPG) units per liter of either Maxilact 20,000 (1.61 g) or Maxilact 40,000 (0.81 g) was suspended in approximately 100 ml of neutralized whey and added to 3.5 liter of whey that had been adjusted to pH 7 with 8N base (NaOH or KOH). The hydrolysis reaction was carried out at $30^{o}C$ under continuous slow agitation with a magnetic stirrer. Ten milliliter aliquots were taken every hour for glucose determination to monitor the hydrolysis reaction. After a given hydrolysis time, the enzyme in the aliquots was inactivated at $90^{o}C$ for 5 minutes.

A calculated amount (4.83 g) equivalent to 9,100 (ONPG) per liter of either lactase LP or lactase N was suspended in approximately 100 ml of whey, and added to 3.5 liters of the whey (pH 4.6-4.65). The hydrolysis reaction was carried out at $45^{o}C$ with continuous slow stirring.

TABLE 1

IDENTIFICATION OF LACTASE ENZYMES BY MANUFACTURERS

Enzyme	Lot No.	Activity[1]	Manufacturer
Maxilact 20,000	112A	20,000 ONPG U/g	G. B. Fermentation Industries Inc.
Maxilact 40,000	60,175	40,000 ONPG U/g	Enzyme Development Corp.
Lactase LP	K7G377X	200,000 Lu/g	G. B. Fermentation Industries Inc.
Lactase N	D-1	200,000 Lu/g	G. B. Fermentation Industries Inc.

[1]One lactase unit (LU) is defined as that quantity of enzyme required to liberate 3.3×10^{-8} moles of o-nitrophenol per minute from ONPG under the conditions of the test (Wallterstein Co. 1972), and one ONPG unit is defined as the quantity of enzyme which forms 1 Mmol ONP per minute under the conditions of the test (Enzyme Development Corp. 1974).

Ten milliliter aliquots were taken every hour for glucose determination to monitor the hydrolysis reaction. After the required hydrolysis time the pH of hydrolyzed whey was adjusted to 7 with 8N base, then heated to 90°C for 5 minutes.

C. DEPROTEINIZATION OF HYDROLYZED WHEY

Batches of whey for hydrolyzed lactose syrup were treated for 9 hours with 2.76 g of Maxilact 20,000 per batch of 6 liters of whey.

After the hydrolyzed whey was heated to 90°C for 5 minutes, it was cooled to 25°C in a cool water bath and centrifuged at 800 x g for 5 minutes. The pH of the supernatant was raised to 9 with dry $Ca(OH)_2$. Centrifugation at 800 x g for 10 minutes was used to sediment the whey proteins with precipitated minerals. The supernatant was separated and the pH was adjusted to 7 with 85% lactic acid.

D. REMOVAL OF COLOR AND MINERALS

Fifteen hundredths per cent of activated charcoal and 0.2% of fuller's earth (Technical fuller's earth powder obtained from Fisher Scientific Co., Atlanta, Ga.) were added to the hydrolyzed deproteinized whey. Continuous stirring with a magnetic stirrer was used for 30 minutes. The slurry was filtered twice through Whatman No. 1 filter paper.

Several cation and anion exchange resins were evaluated for the demineralization process. Amberlite IR-120 (H) CP, particle size 20-50

mesh, strong acid type in H+ form; Amberlite IR-120, particle size 20-50 mesh, strong acid type in the H+ form, medium porosity (obtained from Rohm and Haas Co.); Dowex 50 W x 4, particle size 20-50 mesh strong acid type in the H+ form (obtained from Dow Chemical Co.) and Rexyn 102 H, particle size 100-200 mesh, weak organic acid type, in the H+ form (obtained from Fisher Scientific Co.) were evaluated as cation exchange resins. Amberlite IR-45 AR., particle size 20-50 mesh, weak base type in the OH form; Amberlite IRA-400 AR, particle size 20-50 mesh, strong base type in the Cl form (obtained from Rohm and Haas Co.); Rexyn 201 (OH), particle size 20-50 mesh, strong base organic type in the OH form (obtained from Fisher Scientific Co.) and Dowex 21K, particle size 20-50 mesh, strong base type, in the Cl form (obtained from Dow Chemical Co.) were evaluated as anion exchange resins. Amberlite IRA-400 AR and Dowex 21 K were treated with 1 N NaOH to exchange the ionic Cl form to the hydroxyl form and the excess NaOH was washed out with deionized water. Amberlite IR-120 AR and Dowex 21K gave the best results as cation and anion exchanges.

A 4.5 x 45 cm glass column filled with Amberlite IR-120 AR was used for the cation exchange process with a flow rate of 66 ml/min. This was followed by the anion column, 4.5 x 40 cm filled with Dowex 21 K with a flow rate of 360 ml/minute.

The batch process was carried out in a 4 liter beaker; 150 ml of cation resin in H+ form was added to 2000 ml of decolorized whey serum and stirred continuously for 10 minutes or until pH reached 2.5 $\pm$ 0.5.

The resin was filtered off through Whatman No. 1 filter paper, and 130 ml of the anion resin was added to the filtrate and stirred for 15 minutes or until the pH reached 7.0. The demineralization process was monitored by measuring the pH and determining the glucose and ash content.

E. CONCENTRATION OF HYDROLYZED LACTOSE SYRUP

Two liter batches of hydrolyzed lactose syrup were concentrated at 60^{o}C under reduced pressure (125 Torr maximum). The concentration process was carried to approximately 80% solids which was monitored by refractometer readings. Several batches were concentrated and stored at 4^{o}C until used.

F. APPLICATION

Five gallon batches of vanilla ice cream were made using the concentrated hydrolyzed lactose syrup as a replacement for corn syrup in increments of 25% of the corn syrup.

The ingredients used for making ice cream mixes were: cream 40%, obtained from The University of Tennessee creamery; non fat dry milk solids, spray process grade A, obtained from Dairymen, Inc.; cane sugar, extra fine granulated sugar (Domino) obtained from Amstar Corp.; corn syrup, D. E. 36, total solids 80%, obtained from A. E. Staley Manufacturing Co.; stabilizer, NPL - 56, its ingredients: cellulose gum, monodiglycerides, guar gum, polysorbate 80, carrageenan and whey powder, obtained from National pectin products; annatto color, Hansen's ice cream

color pure vegetable annatto color, obtained from Hansen's laboratories; vanilla flavor, six fold vanilla-vanillin extract, obtained from Nielsen-Massey Vanillas Inc.

Mixes were pasteurized at 72°C for 30 minutes, then homogenized at 2500 Psi (2000 Psi first stage, 500 Psi second stage). The mixes were cooled at 26°C and stored over night in a cooler at 5°C. The mixes were frozen in a Taylor soft-serve freezer to a drawing temperature of -5°C. The frozen mixes were stored in quart containers at -30°C for 3 days before sensory evaluation. The approximate composition of the ice cream mix is shown in Table 2.

G. ANALYTICAL METHODS

Lactose

Lactose content in whey was determined before hydrolysis using the picric acid colorimetric method (Perry and Doan, 1950). A half milliliter of whey was mixed with 99.5 ml of saturated picric acid. The sample was filtered through Whatman No. 1 filter paper. Two milliliters of the filter rate were transferred to a test tube and 1 ml of 25% Na_2CO_3 reagent was added. The color was developed in a boiling water bath for 20 minutes. The tubes were cooled and diluted to 20 milliliters with distilled water. Absorption of a portion of the sample was read on a Bausch and Lomb Spectronic 20 at 520 nm. A blank consisting of 2 ml of picric acid and 1 ml of 25% reagent Na_2CO_3 was used. A standard curve had previously been constructed using standard solutions of lactose in

TABLE 2

COMPOSITION OF THE ICE CREAM MIX

Component	Content %
Fat	12
Milk Solids	11
Cane Sugar	11.25
Corn Syrup[1]	3.75
Stabilizer	0.3
Vanilla Flavor	0.0015
Annatto Color	0.0156

[1]Hydrolyzed lactose syrup replaced the corn syrup in increments of 25%.

1% incremental concentrations from 1 to 6% (w/w). The following regression equation was obtained:

Absorbance y = 0.0231 + 0.0396 X (% lactose).

and the regression coefficient was .998.

Glucose

Glucose was determined in the whey during the enzyme hydrolysis by the glucose oxidase method (Trinder, 1969). A 10 ml aliquot of whey was diluted to 100 ml with distilled water. One tenth ml of diluted whey was mixed with 2.9 ml protein precipitant [10 g $Na_2WO_4 \cdot 2H_2O$, 10 g Na_2HPO_4 and 4 g NaCl dissolved in 800 ml H_2O; about 125 ml of 1 N HCl was added to bring the pH to 3, 1 g phenol was added and the volume was made to 1 liter with H_2O] and centrifuged. One ml of the supernatant was added to 3 ml color reagent [75 ml of 4% Na_2HPO_4, 220 ml water, 5 ml Fermcozyme 452 DM (mixture of glucose oxidase and peroxidase), 300 mg sodium azide and 100 mg 4-amino phenozone were added]. The color was developed at 37°C for 10 minutes. The absorbance of the samples was read immediately on a Bausch and Lomb Spectronic 20 at 515 nm. A standard glucose solution (200 mg/100 ml) was prepared. Standard glucose solution, 0.1 ml, was mixed with 2.9 ml protein precipitant. One ml of the standard mixture and 1 ml of protein precipitant solution (as blank) were treated along with the sample.

The glucose concentration was calculated using the following equation:

$$\text{Glucose} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200 \times \frac{10}{1000}$$

Absorbancies were maintained between 0.30 and 0.50 by proper adjustment of relative concentrations.

Lactose Hydrolysis

The per cent lactose hydrolysis was calculated as follows:

$$\%\ \text{lactose hydrolysis} = \frac{\text{glucose \%}}{\text{original lactose \%}} \times 1.9 \times 100$$

Total Solids

Total solids were determined on unconcentrated hydrolyzed lactose syrup in a vacuum drying oven, method 31.005 (AOAC 1975). Approximately 5 g of hydrolyzed syrup were dried in triplicate on a hot plate to evaporate moisture; the samples were then dried at 60°C for 24 hours at a vacuum of 125 Torr.

Total solids were determined on the concentrated hydrolyzed lactose syrup. Approximately 2 g of the syrup were dried on sand in triplicate as previously described.

Total Solids by Means of Refractometer

Refractive index was read using an Abbe 56 refractometer (Bausch and Lomb) at 45°C according to method 31:199 (AOAC 1975). From tables 31:09 and 31:10 (AOAC 1975) the total solids were obtained to follow the concentration of the hydrolyzed lactose syrup.

Ash

Ash content was determined according to method 31:012 (AOAC 1975). Approximately 10 g of unconcentrated hydrolyzed lactose syrup or 5 g of concentrated syrup were dried on a hot plate and then heated slowly over a Bunsen flame until foaming stopped. The samples were ignited at 525°C until a white ash was obtained (6 hrs.). Determinations were made in triplicate.

Protein

Protein was determined on 5 g of concentrated hydrolyzed lactose syrup by the Kjeldahl method (AOAC 1975). A factor of 6.38 X N was used.

Color

The color of concentrated hydrolyzed lactose syrup was measured by reflectance using an IDL Color Eye Model D-1. A 1 cm cuvette was filled with sample and reflectance measurements were read against a standard white vitrolite tile with the factors:

$X\ (vit) = 0.6835$

$Y\ (vit) = 0.8805$

$Z\ (vit) = 1.0370$

$\bar{X}\ (vit) = 0.1755$

Color eye X, Y, Z and $\bar{X}$ were measured and then converted to CIE x, y, and CIE.

Viscosity

Samples of the ice cream mix were transferred into 100 ml beakers. The beakers were held in a water bath at 10°C. The apparent viscosity of each ice cream mix was measured with a Brookfield LVF Viscometer with spindle No. 2 at 12 rpm. The viscometer was turned on and the spindle was allowed to travel through the sample for 1 minute. The viscometer was stopped and the reading was recorded. The readings were converted to centipoises using the conversion factor for the spindle size and the rotational speed.

H. SENSORY EVALUATION

Samples of ice cream in one quart containers held at -30°C were transferred to storage cabinet at -15°C for 3 hours before the time of sensory evaluation. The containers were cooled and presented to an expert panel of 5 experienced judges of dairy products. The standard American Dairy Science Association ice cream score and (Appendix) was used to evaluate flavor, body and texture of the samples.

I. STATISTICAL ANALYSIS

Analytical and sensory evaluation data were analyzed by appropriate analysis of variance procedures.

CHAPTER IV

RESULTS AND DISCUSSION

A. EVALUATION OF LACTASE ENZYME ACTIVITIES

The method employed for the evaluation of the activities of the four enzymes for the hydrolysis of lactose in whey was based on determining the amount of glucose accumulated in the hydrolysis process. The enzymes used were Maxilact 40,000, Maxilact 20,000, Lactase LP and Lactase N. They varied in their absolute activities, their sources, and methods of preparation. The amounts used of each of the four enzymes were calculated to correspond to 9100 ONPG units per liter. This amount of enzyme should induce a maximum hydrolysis activity under predetermined optimum hydrolysis conditions.

Maxilact 40,000 and Maxilact 20,000 are prepared from Saccharomyces spp yeast. Their activities are 40,000 and 20,000 ONPG U/g, respectively, with the conditions of maximum activity, 30^{o}C and pH 7.0 (Enzyme Development Corp., 1974). On the other hand, both Lactase LP and Lactase N are prepared from Aspergillus niger. Each was claimed (Wallerstein Co. 1974) to have the same enzyme activity of 200,000 LU/g (equal to 6600 ONPG U/g), with the conditions of maximum activity at 37 - 55^{o}C and pH of 3.8 - 4.6.

Maximum activity of Maxilact enzymes is achieved at a neutral pH. For this reason the whey was adjusted to pH 7 before addition of the enzymes. Two alkaline solutions, potassium hydroxide and sodium

hydroxide, were initially used to neutralize the acidic whey. An 8 N solution of each of these alkalis was added to the whey and its effect on the enzyme activity was investigated. Table 3 shows that Na+ ions had an adverse effect on the Maxilact activity, while KOH gave higher values of lactose hydrolysis. In the case of KOH the gain in activity over that corresponding to NaOH was in the range of 30%. This agreed with Dahlqvist et al. (1977) who indicated that the activity of the Saccharomyces lactis lactase is higher in the presence of K^+ than of Na^+. Therefore, KOH was chosen to neutralize the whey.

Three independent lactose hydrolysis replications were performed. The mean values of lactose hydrolysis (per cent) of the four enzymes in these three replications is shown in Table 4 as a function of time.

After a one hour period of hydrolysis both Lactase LP and Maxilact 40,000 apparently showed superior activities when compared to Lactase N and Maxilact 20,000. This superiority diminished with time and the differences in activities among the four enzymes became increasingly smaller. At the end of 7 hours, over 90% of the initial lactose in the whey was hydrolyzed by the four enzymes.

Although complete hydrolysis of lactose in whey is possible from a theoretical view point, experimentally, a complete hydrolysis cannot be achieved. The maximum hydrolysis reached in this study was 94.80% with Lactase LP. This experimental limitation can be explained through the realization that the lactose enzymatic reaction is not limited to a simple

TABLE 3

EFFECT OF ALKALI SOURCE ON THE ACTIVITY OF MAXILACT 40,000 IN WHEY

Alkaline soln. used to neutralize the whey to pH 7[2]	Lactose hydrolysis[1]		
	Rep 1	Rep 2	Rep 3
	%		
KOH 8N	92.70	92.50	92.57
NaOH 8N	62.25	64.79	65.00

[1]Initial lactose content 4.6%, hydrolysis time 3 hours.

[2]Initial whey pH 4.6 - 4.65.

TABLE 4

LACTOSE HYDROLYSIS OVER TIME BY FOUR ENZYMES

Time of Hydrolysis (hr)	Lactose Hydrolysis[1]			
	Maxilact 40,000	Maxilact 20,000	Lactase LP	Lactase N
	%			
1	50.75	35.04	52.50	48.67
2	65.75	61.95	69.18	63.04
3	90.39	77.07	80.12	71.44
4	91.05	80.58	92.00	81.83
5	92.20	84.97	93.27	88.04
6	93.61	88.26	94.53	89.92
7	93.99	91.86	94.75	91.61
8	93.99	92.49	94.80	93.39
9	93.99	93.38	94.80	93.90

[1]Means of three replications.

hydrolysis with the production of glucose and galactose, but is accompanied by the immediate formation of other oligosaccharides (Aronson, 1952). Also, it has been reported that the presence of galactose inhibits the enzymatic hydrolysis of lactase (Webb et al., 1974).

Figure 1 illustrates graphically lactose hydrolysis by the four enzymes related to time. These curves are characterized by an initial rapid increase of the hydrolyzed lactose followed by an exponentially decreasing rate until a final asymptotic value of 92 - 94% is reached. It is clear that the relationship between each enzyme activity and time is nonlinear in nature. The initial mathematical model used to express this relationship was a polynomial with hydrolysis time as the independent variable and per cent hydrolyzed lactose as the dependent variable. A better fit to the experimental data was found to be of the general exponential form:

$$\text{Hydrolyzed lactose per cent} = b - b\, e^{-kt}$$

t = hydrolysis time in the range 1 to 9 hrs.

a, b and k are constants whose value depends on the enzyme used.

The constant k is inversely proportional to the time constant of reaction. The larger the value of k the faster the hydrolyzed lactose percentage reaches its final asymptotic value (which is a). The value of bk defines the initial rate. Values of the constants a, b and k for the four used enzymes are shown in Table 5.

Among the four enzymes studied, Maxilact 40,000 is associated with the highest initial rate. It reached a value of 90.39% in about 3 hours. On the other hand, Lactase N is the slowest of the four enzymes

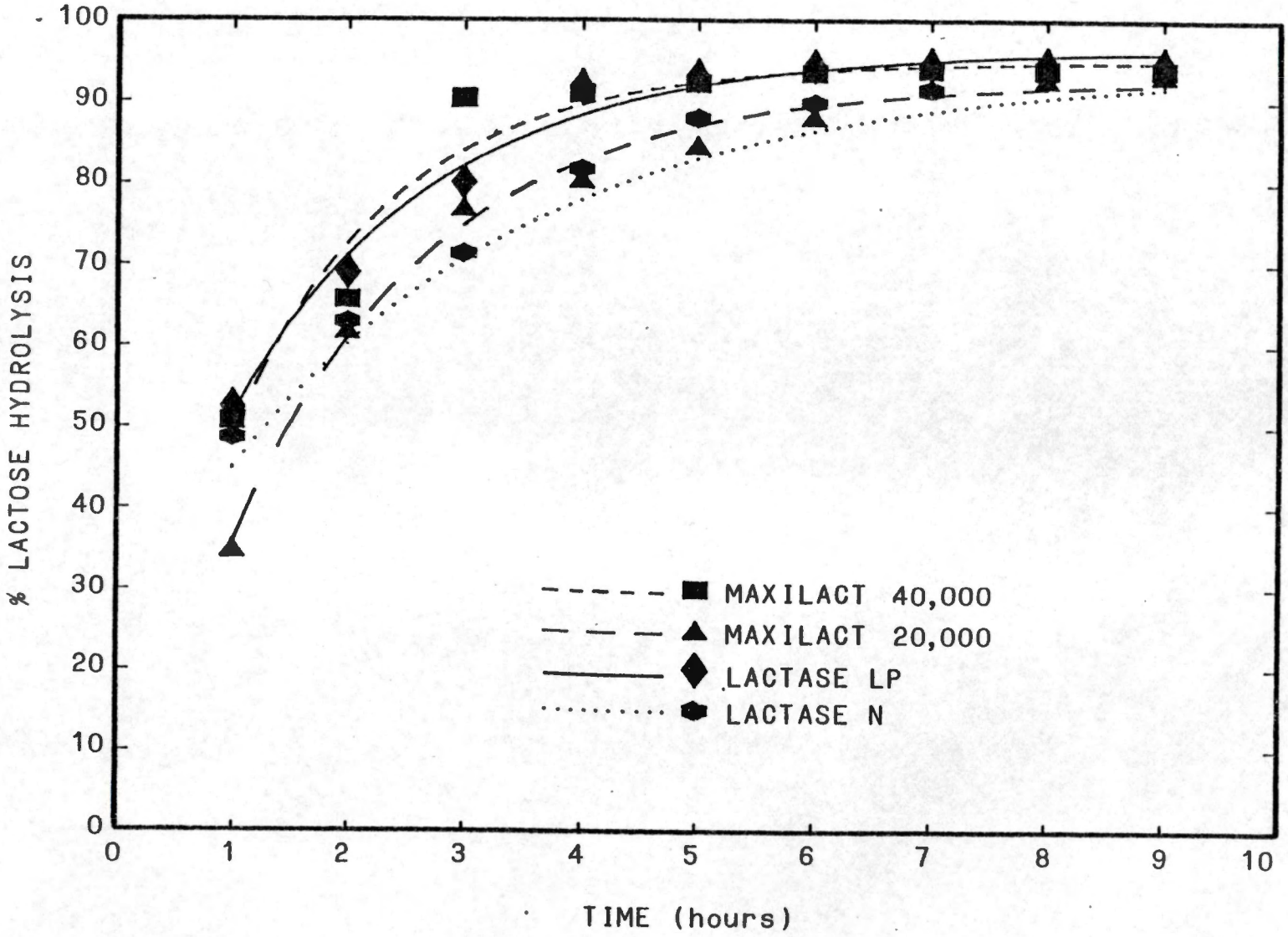

Figure 1. Lactose hydrolysis over time for the four enzymes.

TABLE 5

LACTOSE HYDROLYSIS EXPONENTIAL MODELS

Enzyme	Model Constants[1] a	b	k	Initial Rate (bk)	X(9)
Maxilact 40,000	95.0735	94.0286	0.7146	67.19	94.9221
Maxilact 20,000	92.6135	101.8284	0.5823	59.29	92.0742
Lactase LP	96.5532	79.5451	0.5696	45.31	96.0809
Lactase N	97.1403	71.4094	0.3723	26.59	94.6367

[1] Model $x(t) = a - bc^{-kt}$
cal.

in this initial phase. At the end of the 9 hour period (Table 4, page 33) the hydrolyzed lactose percentage of the four enzymes had almost reached their asymptotic values. Over the 9 hour period of hydrolysis, Lactase LP gave the highest mean value of hydrolysis (94.80%) while Maxilact 20,000 gave the lowest (93.38). However, that these two values represent a relatively narrow displacement between the highest and the lowest final hydrolyzed lactose percentage.

It was noticed that the hydrolyzed whey with Lactase N had undesirable properties such as dark color and an objectionable odor. The undesirable odor might be caused by a high content of proteases in Lactase N. Therefore, Lactase N usage was avoided.

From an economical viewpoint, Maxilact 20,000 represented a good choice for use in whey hydrolysis in the production process of hydrolyzed lactose syrup. The relatively long time required to reach the maximum hydrolysis by this enzyme may turn out to be more convenient in case of industrial application. The whey resulting from cheese manufacture in a cheese plant could be collected on a daily basis. The hydrolysis process then could be carried out overnight in 8 to 9 hours. This interval could be quite sufficient for maximum hydrolysis by Maxilact 20,000.

Although the proposed time for a maximum hydrolysis may be considered disadvantageous due to the liklihood of contamination, it was noticed that the whey at the end of this hydrolysis time did not have a pH lower than 6.4. This indicates only a limited growth of microorganisms in the whey. This growth is limited and virtually eliminated by later

stages of the production such as heating the hydrolyzed whey to 90°C for 5 minutes, centrifugation, decolorization process, and filteration. These steps prevent any consequences that may be caused by the growth of these microorganisms during the hydrolysis period.

Lactase LP achieves its maximum activity at pH 4.6 which is unfavorable for undesirable microorganisms. However, this enzyme is more expensive when compared to Maxilact 20,000 and it is not currently available on the market.

B. DEMINERALIZATION OF THE HYDROLYZED WHEY

The hydrolyzed whey was heated to 90°C for 5 minutes in order to denature the whey proteins, which were removed by centrifugation.

Wierzbicki and Kosikowski (1973c) indicated that treating the whey with sodium hydroxide to pH 9.0 precipitated protein and minerals. The protein with entrapped minerals was removed by centrifugation, then the pH was adjusted to 7 by ion exchange or addition of lactic acid. They reported that the concentrated syrup resulting from this process was very sweet without taint or saltiness. But when this procedure was applied in the present study, the deproteinized and demineralized product was still salty. Sodium hydroxide was replaced with calcium hydroxide (powder) in order to minimize the sodium ions in an effort to decrease the salty taste. Also the solubility of calcium lactate, resulting from the neutralization process with lactic acid, is lower than that of sodium lactate. Precipitated calcium lactate can be removed by filtration during the decolorization process. Part of the remaining residual minerals were removed by ion exchange following the decolorization process.

Several ion exchange resins (listed in Chapter III) were investigated for their efficiencies for whey demineralization. Preliminary experiments were conducted to evaluate the ion exchange resins on the basis of removal of salts and sugar loss in the hydrolyzed deproteinized whey.

Amberlite IR - 120 AR as a cation exchange resin and Dowex 21 K as an anion exchange resin gave a minimum sugar loss, about 3%, while some of the other resins evaluated gave about 50% loss.

Table 6 shows the experimental results associated with the use of Amberlite IR - 120 AR and Dowex 21 K in three different batches of whey. The demineralization process was carried out by the batch method.

pH values before and after treatment with each resin are shown for the three replications. Glucose and ash percentage as well as the relative loss in each are also reported. The relative loss in glucose did not exceed 3.2% while that of ash was approximately 80%. It is important to note that the small amount of remaining ash content did not affect the palatability of the demineralized hydrolyzed lactose syrup. Moreover, the lower the pH after the treatment with cation resin, the lower was the ash content of the final product. For later work, the cation exchange reaction time was stopped after 10 minutes irrespective of the exact final pH value, as long as it fell in the range of 2.5 $\pm$ 0.5.

TABLE 6

DEMINERALIZATION OF HYDROLYZED WHEY BY ION EXCHANGE RESINS

Replications	pH Cation[1] before	pH Cation[1] after	Anion[2] before	Anion[2] after	Glucose[3] before	Glucose[3] after	Glucose[3] loss	Ash[4] before	Ash[4] after	Ash[4] loss
						%				
1	6.95	2.00	2.00	7.00	2.47	2.42	2.02	11.63	2.29	80.31
2	7.00	2.40	2.40	7.00	2.55	2.47	3.14	12.59	2.58	79.51
3	7.00	2.20	2.20	6.95	2.50	2.42	3.20	13.17	2.69	79.57
Mean ± S.D.	6.98 ± 0.029	2.2 ± 0.2	2.2 ± 0.2	6.98 ± 0.029	2.51 ± 0.040	2.44 ± 0.029	2.79 ± 0.665	12.46 ± 0.778	2.98 ± 0.65	79.80 ± 0.45

[1]Cation resin: Amberlite IR - 120AR

[2]Anion resin: Dowex 21K

[3]Glucose concentration on wet basis, means of duplicate measurements

[4]Ash content as % of total solids, means of triplicate measurements.

C. HYDROLYZED LACTOSE SYRUP PROPERTIES

Hydrolyzed lactose in whey passed through a sequence of processes that started with deproteinization followed by decolorization and partial demineralization. The final process was concentration which was performed at 60°C under a reduced pressure that did not exceed 125 Torr. Idealized concentration of solids, at these conditions, in the clarified hydrolyzed whey should result in a pure water evaporation with only minimal change in the composition or character of the solids in the syrup. However, the actual evaporation process may have included substantial changes in the chemical and physical nature of the processed syrup. One of the most important of these changes is that of color, which influences the syrup quality. Maillard browning reaction may occur during the concentration process as a result of the reaction between the protein residue in the syrup and the reducing sugars. Measuring the color of the hydrolyzed lactose syrup was necessary to determine the extent of the browning reaction in the concentrated syrup. Table 7 shows the results of CIE color measurement of four different batches of hydrolyzed lactose syrup. The attributes of special color, saturation and lightness are designated by x, y and YCIE. YCIE denotes "lightness." The analysis of variance of CIE color parameters of 4 different batches prepared similarly (Table 8) showed no significant difference among the batches or the samples with respect to x and y, while there was a significant difference at $P < 0.05$ among the batches for the parameter

TABLE 7

CIE COLOR OF HYDROLYZED LACTOSE SYRUP

Batches	x	y	YCIE[1]
1	0.35	0.36	29.62[a]
2	0.36	0.37	29.82[a]
3	0.35	0.36	28.83[b]
4	0.35	0.36	28.51[b]

[1]Means in columns followed by the same letter are not significantly different at the 5% level, n = 3.

TABLE 8

MEAN SQUARE VALUES OF CIE COLOR PARAMETERS AND SIGNIFICANCE OF F-RATIOS OF HYDROLYZED LACTOSE SYRUP

Source	df	Mean Square		
		x	y	YCIE
Batches	3	0.00003	0.00003	1.1692*
Samples	2	0.000025	0.0000	0.0447
Batches x samples (Residual)	6	0.000025	0.00003	0.0968
TOTAL	11			

*P<0.05.

YCIE. Since the YCIE value indicates the lightness of the syrup which is associated with the luminous energy reflected by the syrup sample, the differences among the batches in this parameter can be attributed to experimental processes such as deproteinization, decolorization, demineralization and particularly concentration conditions.

Several batches of hydrolyzed lactose whey with 92.98 $\pm$ 0.69% of the lactose hydrolyzed were concentrated and pooled to provide enough hydrolyzed lactose syrup for application. Composition and properties of the pooled hydrolyzed lactose syrup are shown in Table 9. The Munsell system of color notation was calculated from the CIE parameters x, y and YCIE. The Munsell notation of the hydrolyzed lactose syrup denotes a yellow hue (7.5 y) that is moderate in value (5.83 /) and moderately strong (/12.2) in chroma. The Munsell hue, value and chroma were determined from its daylight reflectance, Y, and chromaticity coordinates, x, y, in the CIE system by reference to the scales of the ideal Munsell system (A S T M, 1968).

Apparent viscosity of the pooled hydrolyzed lactose syrup is recorded in Table 9. Since the viscosity of a liquid is often of great importance in determining the characteristics of a food product, the viscosity of the syrup can be related to its consistency.

D. APPLICATION OF HYDROLYZED LACTOSE SYRUPS

The prepared hydrolyzed lactose syrup was used as a replacement for corn syrup (3.75% by wt) in vanilla ice cream. The per cent of

TABLE 9

COMPOSITION AND PROPERTIES OF HYDROLYZED LACTOSE SYRUP IN ICE CREAM

Composition[1]:	
Ash =	2.42%
Protein =	2.77%
Total solids =	80.97%
Refractive Index[1] =	1.4858
Color[1]	
x =	.35
y =	.36
yCIE=	28.51
Munsell Notation =	7.5 y - 5.83 / 12.2
Apparent viscosity[1,3]	2050 cps @ 25°C
Degree of hydrolysis[2]	92.98% ± 10.69 of initial lactose

[1]Means of triplicate measurements on one pooled lot of syrup.

[2]Mean of 14 batches ± 1 standard deviation.

[3]Brookfield viscometer (LVF), spindle No. 2, 12 rpm at 25°C.

hydrolyzed lactose syrup replacement in ice cream mix was varied between 0 to 100% in increments of 25% (Appendix). Viscosity of the ice cream mix was taken as one means of indicating the effect of replacing corn syrup by hydrolyzed lactose syrup. Viscosity has been considered an important property of ice cream mix (Arbuckle, 1972). The means of three independent apparent viscosity measurements in each of two replications are shown in Table 10 as a function of corn syrup replacement. The apparent viscosity decreased with increased syrup replacement. This behavior was expected since the apparent viscosity of corn syrup is higher than that of the hydrolyzed lactose syrup. The analysis of variance and Duncan's multiple range tests showed a significant difference among the different mixes in the two replicates. The apparent viscosity decreased significantly with increased syrup replacement. Analysis of variance (Table 11) showed also significant difference between the two replicates. This difference may be caused by difference in handling or processing the mix in the two replicates. Pasteurization, homogenization and aging have the greatest effect on the apparent viscosity of the ice cream mix (Leighton et al., 1934).

E. SENSORY EVALUATION

The results of taste panel assessments are presented in Table 12. As shown, there was no significant change in flavor or in body and texture with increased percentage of hydrolyzed lactose syrup. Statistical analysis (Table 13) indicated that increasing the percentage of replacement

TABLE 10

APPARENT VISCOSITY FOR ICE CREAM MIXES AS A FUNCTION OF HYDROLYZED LACTOSE SYRUP IN ICE CREAM

Syrup replacements %	Rep I[1]	Rep II[1]
	Centipoise	
0	230.00[a]	241.66[a]
25	217.33[b]	230.90[b]
50	212.66[b]	221.33[b]
75	209.76[b]	215.16[c]
100	185.00[c]	195.53[d]

[1]Means in columns followed by the same letter are not significantly different at the 5% level, n = 3.

TABLE 11

MEAN SQUARE VALUES FOR APPARENT VISCOSITY AND SIGNIFICANCE OF F-RATIOS OF ICE CREAM MIXES

Source	df	Mean square
Treatments	4	1702.3704*
Replication	1	745.0287*
Replication x treatment	4	14.5132
Sample	2	9.8665
Treatment x sample	8	3.9238
Residual	11	4.2603
Total	30	

*P<0.05.

TABLE 12

SENSORY EVALUATION OF ICE CREAM

% Syrup Replacement	Flavor[1]		Body and Texture[2]			
			Replicate I		Replicate II	
	Mean	Range	Mean	Range	Mean	Range
0	7.75	6-9.5	2.2	1-3	4.2	4-4.5
25	8.30	7-9	2.6	2-3	4.1	3-5
50	7.80	7-9	3.0	3	3.1	2-4
75	7.40	6-9	2.6	2-3	3.7	3-4
100	7.45	5-9	2.4	2-3	3.8	3-4

[1]Numerical values: perfect flavor = 10, poor flavor = 1, n = 5.

[2]Numerical values: perfect texture = 5, poor texture = 1, n = 5.

TABLE 13

MEAN SQUARE VALUES AND SIGNIFICANCE OF F-RATIOS OF SENSORY EVALUATION

Source	df	Mean Square	
		Flavor	Texture
Syrup	4	1.3	0.21
Replications	1	8.82	15.125*
Syrup x replications	4	1.63*	1.29*
Panelists	4	2.93	0.895
Residual	36	0.55	0.22
Total	49		

*$P<0.05$.

of corn syrup with hydrolyzed lactose syrup did not affect either flavor or the body and texture of the ice cream ($P<0.05$). However, there was a significant difference between the two replicates with regard to body and texture. The reason for this difference was the difference in the freezing time. The first replicate took a longer time than the second replicate to reach to the drawing temperature ($-5^{o}C$) which affected the body and texture by causing churning of the fat in the mix.

CHAPTER V

SUMMARY

The objective of this study was to prepare a hydrolyzed lactose syrup from cottage cheese whey and use it as an ingredient in ice cream mix.

Four lactase enzymes, Maxilcat 40,000, Maxilact 20,000, Lactase LP and Lactase N were evaluated for their lactose hydrolysis activities. Over a 9 hour period of hydrolysis, Lactase LP gave the highest mean value of hydrolysis (94.80%), Maxilact 40,000 gave 93.99% lactose hydrolysis, Lactase N gave 93.90% and Maxilact 20,000 gave only 93.38%. However, Maxilact 20,000 was chosen because of economical and convenient industrial application for the hydrolysis process in this study.

The hydrolyzed whey was heated to 90^{o}C for 5 minutes followed by centrifugation in order to remove the denatured whey proteins. The color was removed by treating the hydrolyzed deproteinized whey with 0.15% charcoal and 0.2% fullers earth. The demineralization process was carried out with ion exchange method. Amberlite AR-120 AR as a cation and Dowex 21K as an anion exchange were used to reduce the mineral content of the hydrolyzed whey. Residual ash was reduced to less than 2.5%.

The clarified whey was concentrated at 60^{o}C under reduced pressure to 80% total solids. The properties of the concentrated hydrolyzed lactose syrup were determined.

The hydrolyzed lactose syrup was used as a replacement for corn syrup (3.75% by wt) in ice cream. Replacement level was varied between 0 and 100% of the corn syrup in increments of 25%. Increasing levels of hydrolyzed lactose syrup decreased the apparent viscosity significantly.

Results of an expert taste panel indicated that replacing the corn syrup with hydrolysed lactose syrup did not affect significantly either the flavor or the texture and body of the ice cream.

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APPENDIX

DATE_____ A.D.S.A.

CONTEST ICE CREAM SCORE CARD

D.I.S.A._____CONTESTANT No._____

PERFECT SCORE	CRITICISMS	SAMPLE NO 1	2	3	4	5	6	7	8	9	10	TOTAL GRADES
FLAVOR 45	CONTESTANT SCORE ➧											
	GRADE SCORE											
	GRADE CRITICISM											
	COOKED											
	LACKS FLAVORING											
	TOO HIGH FLAVOR											
NO CRITICISM 10	UNNATURAL FLAVOR											
	HIGH ACID											
	LACKS FINE FLAVOR											
	LACKS FRESHNESS											
	METALLIC											
	OLD INGREDIENT											
NORMAL RANGE 1–10	OXIDIZED											
	RANCID											
	SALTY											
	STORAGE											
	LACKS SWEETNESS											
	SYRUP FLAVOR											
	TOO SWEET											
BODY AND TEXTURE 30	CONTESTANT SCORE ➧											
	GRADE SCORE											
	GRADE CRITICISM											
NO CRITICISM 5	COARSE/ICY											
	CRUMBLY											
	FLUFFY											
NORMAL RANGE 1–5	GUMMY											
	SANDY											
	SOGGY											
	WEAK											
COLOR 5	ALLOWED PERFECT IN CONTEST ➧	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
MELTING QUALITY 5	ALLOWED PERFECT IN CONTEST	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
BACTERIA 15	ALLOWED PERFECT IN CONTEST	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	
TOTAL 100	TOTAL SCORE OF EACH SAMPLE ➧											
	TOTAL GRADE PER SAMPLE											

	Code	Grade
TEAM RANK	1	
	2	
	3	
	TOTAL	
	RANK	

FINAL GRADE _____

RANK _____

TABLE 14

THE CALCULATED CONSTITUENTS OF THE ICE CREAM MIXES

Ingredient	I[a,b]	II[a,b]	III[a,b]	IV[a,b]	V[a,b]
	Kg				
Cream 40% Fat	6.15	6.15	6.15	6.15	6.15
Non fat dry milk	1.98	1.98	1.98	1.98	1.98
Sucrose	2.30	2.30	2.30	2.30	2.30
Corn syrup 80%	0.96	0.72	0.48	0.24	0
Hydrolyzed lactose syrup 80%	0.96	0.72	0.48	0.72	0.96
Stabilizer	0.062	0.062	0.062	0.062	0.062
Water	9.05	9.05	9.05	9.05	9.05
TOTAL	20.50	20.50	20.50	20.50	20.50

[a]I. 100% corn syrup, 0% hydrolyzed lactose; II. 75% corn syrup, 25% hydrolyzed lactose; III. 50% corn syrup and 50% hydrolyzed lactose; IV. 25% corn syrup, 75% hydrolyzed lactose; V. 0% corn syrup, 100% hydrolyzed lactose.

[b]30 g of vanilla flavor and 3.0 g of annato color were added to each batch.

VITA

The author was born in Cairo, Egypt, on October 27, 1940 to Mr. and Mrs. Mohamed Tawfik El-Hefnawy. He attended elementary school and high school in Cairo, Egypt, and was graduated in 1960. He attended the University of Ein-Shams at Cairo and in June, 1965, was graduated with Bachelor of Science degree in Food Science. In October of the same year, he was employed as a Food Technologist by Kafr El-Zayat Company (currently Soap and Oils Co.), Alexandria, Egypt. In 1966, the author began a graduate program at Alexandria University, Department of Agricultural Industries. In July 1968, he transferred to El-Nasr Tobacco and Cigarettes Co., as a Quality Assurance Technologist. In January 1972, he received a Master of Science degree with a major in Dairy Science. In the same year, he was promoted to a research associate in Research and Development in the same company at Cairo. In September 1972, he entered Cairo University to initiate work toward the Ph.D. in Biochemistry. In 1975, he was promoted to Senior Research Chemist in the same company. In 1976, he came to the United States for practical training on the redrying and processing of tobacco. In the summer of the same year, he entered The University of Tennessee and continued work toward the Doctor of Philosophy degree. In November 1976, he accepted part time work in Tom's Foods LTD at Knoxville as a research assistant. In December 1978, requirements for the Doctor of Philosophy degree with a major in Animal Science and an

option in Food Technology and Science were completed. He is a member of the Institute of Food Technologists and American Dairy Science Association.