PREPARATION OF HYDROLYZED LACTOSE SYRUP FROM WHEY PERMEATE AND ITS FUNCTIONAL PROPERTIES IN WHITE PAN BREAD FORMULATION

by

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INTRODUCTION

The future of the dairy industry looks brighter on the account that it is able to maximize the utilization of its by-products. There are significant increases in the production of by-products of cheese and related products. However, underutilization of these by-products remains a major concern of the industry. The advent of high technology such as the ultrafiltration process (UF) and immobilized enzyme (IME) has opened the opportunity for the dairy industry to produce quality food ingredients such as whey protein concentrate (WPC) and hydrolyzed lactose syrups (HLS) from whey and whey permeates. These by-products are generally underutilized inspite of their higher potentials and fuctionalities. For example, researchers in recent years have made HLS from whey and whey permeates and have attempted to use it in ice cream and dairy drink mixes. However, the use of this functional ingredient in baked products has yet to be reported as far as we understand, although the bakery industry has been a strong dairy ingredient user in the past.

Therefore, the objectives of this study were to:

1. immobilize G-galactosidase on a solid support,
2. use the immobilized enzyme to hydrolyze whey permeates,
3. classify and prepare the hydrolyzed permeate into variables,
4. process each of the variables by deionizing and decolorizing,
5. concentrate the variables to syrups,
6. use the hydrolyzed lactose syrups in white pan bread formulation,
7. evaluate the doughs and breads made from the hydrolyzed syrups and observe some specific physical and chemical characteristics,
8. conduct in-house sensory evaluation on the breads.

Whey permeate was obtained from the Kansas State University Dairy Processing Plant (Manhattan, KS) and used as the substrate for the enzymatic hydrolysis process using an immobilized enzyme system. The hydrolyzed whey permeates were divided into three variables of different degrees of lactose hydrolysis. Each variable was then classified as "demineralized" and "undemineralized" and processed accordingly. All the variables were decolorized and concentrated to syrups. The syrups were used in white pan bread formulation at 6% flour basis (700g flour) to substitute sucrose. Sucrose was used as the control. The breads were made in triplicate batches.

REVIEW OF THE LITERATURE

I. Whey Permeate: General Outlook

Whey permeate is the watery liquid by-product of ultrafiltration (UF) process of whey and /or milk. It is largely made up of lactose, ash and traces of non-protein nitrogenous materials (Chandan, 1982; Hausser, 1984). Approximately 90% of whey is whey permeate with its contents estimated at about 85% lactose, 9% ash and 4% non-protein materials on a dry weight basis (Chandan, 1982).

In 1983, American dairy farmers produced about 140 billion pounds of milk and approximately one-third of this was processed into cheese. This represents 5 billion pounds of cheese and 45 billion pounds of whey (Luksas, 1984). The UF process which fractionates the whey solids from whey, especially its protein, results in the production of whey permeate. Thus, it is known that most permeate on the market today comes from cheese manufacturing, especially from sweet cheeses such as Cheddar cheese.

Precise data on whey permeate production is not easy to obtain at present time. This is because the Whey Product Institute (WPI), the trade association that collects data on all whey related products have no data available on whey permeate (WPI, 1984). However, the pattern of whey permeate production can be traced alongside with whey. Luksas (1984) reported production figures of 45 billion pounds of whey in 1983 in the U.S. alone. In another report by the International Dairy Federation (IDF), the world cheese output in 1983 was 10 million metric tons for an estimated 85 million metric tons of liquid whey (Zall, 1984). This figure represents an ocean of whey that will result in equally large supply of whey permeate. The address of Horton (1982) provided a direct figure regarding whey permeate in

the U.S. He estimated the U.S. daily production of permeate at 20 million pounds; this estimation was similar to that of Elmer and Clark, Jr. (1982).

All these figures indicate that enormous volumes of whey and whey products, including whey permeate, are currently in production. This warrants a need for the whey industry to find better alternatives for the utilization of these by-products. In order to better utilize these by-products, it is also important that one knows and understands what each of them is made of and how these components function chemically or physically.

II. Whey Permeate Composition

There are many factors influencing the composition of permeate. Among these are: milk production, type of process and operating conditions used to remove the protein, and the analytical methods for detecting and analyzing such components (Hobman, 1984). However, the composition of sweet permeates are essentially similar, if considered on dry basis (Hobman, 1984). It usually consists of lactose as the major solids, minerals and ash, total nitrogen and non-protein nitrogen. An acid type whey, in addition to the components mentioned above, will also contain residual lactic acids (Hobman, 1984; Ennis and Higgins, 1982; Zadow, 1984). See Tables 1A and 1B for typical composition.

A. Lactose

Lactose is the main carbohydrate in milk (Alm, 1982). It accounts for approximately 90% of the total solids (TS) in deproteinated milk serum (DPMS) and about 76% of the total solids in acid-type whey permeate (Hobman, 1984). It is a reducing sugar, a disaccharide that is hydrolyzable to two monsaccharide units, glucose and galactose (Figure 1). Its hydrolysis in

Table 1A - Typical Gross composition (%, wt/vol) and mineral composition (%, wt/vol) for ultrafiltration-derived deproteinated milk serum (DPMS). Gross composition data represent means (and standard deviations) from a variety of equipment[a]

Component	Whole milk		Skim milk		Cheddar cheese whey		Lactic casein whey	
	X	SD	X	SD	X	SD	X	SD
Total solids	5.60	.23	5.77	.19	6.41	.15	5.97	.45
Lactose (monohydrate)	5.03	.20	5.06	.31	5.80	.23	4.55	.45
Total nitrogen	.052	.02	.060	.015	.047	.009	.062	.011
Non-protein nitrogen	.032	.008	.023	.006	.036	.005	.042	.006
Mineral (ash)	.46	.02	.47	.02	.54	.05	.74	.05
Lactate								
Calcium	.03		.02		.05		.62	
Sodium	.12		.06		.06		.14	
Potassium	.01		.16		.18		.17	
Magnesium	.01		...		.01		.07	
Phosphate (total)								
Chloride	.10		.12		.15		.11	

[a]Adapted from Hobman (1984).

Table 1B - Typical compositions of whey permeate, skim milk permeate, and whole milk permeate.[abd]

Product	Na	Ca	Mg	K	Ash	NPN[c]	Lactose
Skim milk permeate	1.01	.43	.11	2.36	10.0	3.43	84
Whole milk permeate	.84	.48	.12	1.99	8.4	3.30	84
Cheddar cheese whey permeate	1.12	.70	.15	2.74	10.2	3.57	86
Whey	.75	.70	.12	2.57	5.0	3.30	77

[a]Averages of number of trials, with different equipment under different conditions.

[b]Percent (wt/wt) on dry matter.

[c]NPN = Non-protein nitrogen.

[d]Adapted from Zadow (1984).

Table 2 - Relative sweetness and solubility of some common saccharides.

	Relative sweetness	Solubility (g/100 g solution)		
		10°C	30°C	50°C
Sucrose	100[a]	66[b]	69[b]	73
Lactose	16[a]	13[b]	20[b]	30[b]
D-Galactose	32[a]	28[b]	36[b]	47[b]
D-Glucose	74[a]	40[b]	54[b]	70[b]
D-Fructose	173[a]	. .	82	87

[a]Pazur (1970).

[b]Shah and Nickerson (1978).

the food industry is mainly by immobilized enzyme technology using the carbohydrolase called ß-galactosidase.

Lactose exists in two isomeric forms, alpha and beta. The isomers are different in the configuration of the substituents on the number one carbon atom of glucose residue (Nickerson, 1974; Zadow, 1984). The isomers also exhibit different solubility property with the alpha showing solubiltiy of 7g/100g at 15°C and beta 50g/100g (Zadow, 1984).

Apart from the poor solubility of lactose, it is also not a very sweet sugar in comparison with other common sugars (Table 2). It forms crystals which give undesirable body defects in frozen dairy desserts (defect known as sandiness in ice cream). Lactose Intolerance is the abnormality associated with the inability to digest lactose when consumed in the diet. It is found to be common among the non-caucasian groups. Lactose, however, has some useful applications. It is known to be a good water binder (humectancy property) and an ideal flavor carrier, and believed to be active in the brown color formation reaction in bread crust.

B. Minerals and other Components

The remaining solids in whey permeate, besides lactose, are primarily nitrogen (mostly the non-protein nitrogen), mineral (mainly calcium and phosphate ions) and lactic acid in decreasing order (Hobman, 1984). The mineral content of permeate are undesirable and must be removed. Presence of mineral residues in large concentrations can lead to ligand (enzyme) inactivation. Besides, minerals can form complexes with the substrate and the reagents used in the immoolized system. Their removal is usually through electrodialysis and/or ion-exchange.

III. Whey Permeate Problems

The whey permeate problems were those essentially inherited from whey. Whey is described as the fluid portion or serum obtained by separating the coagulum from whole milk, cream or skim milk (Zall, 1984). It is a by-product of conventional cheese-making or casein manufacture (Kosikowski, 1979). Underutilization of whey is a major problem facing the whey industry; it is a problem compounded by the increase in the produciton of whey. In addition to the overproduction problem there are additional concerns such as disposal problems and technical understanding of functional properties and nutritional matters of whey and whey products. Inspite of all these problems, whey and its by-products can be effctively processed and utilized in many industrial applications such as pharmaceuticals, animal feed, and human foods.

A. Production vs. Utilization

Horton (1982), in his "Permeate Up-date", estimated that about 20 million pounds of whey permeate are currently produced in the U.S. per day. Other sources, such as the IDF and WPI data on whey production, both in the U.S. and around the world, suggest overproduction at the expense of utilization. Zall (1984) estimated that more than 40% of whey produced in the U.S. is disposed as wastes.

B. Disposal

The water pollution legislation of the 1960's have empowered the government regulatory agencies and municipal governments around the nation to step up their campaign against indiscriminate dumping of industrial discharge into the sewage system. They have issued strong warnings and have

passed into law with tougher measures that will punish severely any unlawful disposals. This type of regulatory action has made it necessary for an industry such as the dairy industry to find an alternative way of treating and handling its waste materials. About 40% of whey produced are unutilized. These are often emptied into the sewage system. Now that this can no longer be practiced without stiffer retribution, the more logical way to go will be to further process the whey into other by-products such as whey permeate and whey protein concentrate rather than the whey treatment program which is very costly.

The biochemical oxygen demand (BOD) of whey is known to be very high. Harper et al. (1971) and Kosikowski (1978) quoted whey's BOD to be about 32,000 ppm or higher. Hobman (1984) estimated it at 35,000 mg/liter. Considering the high volume of whey produced and the subsequent high BOD level, hardly can any municipals allow its sewage to be loaded with such heavy pollutants. Most municipal governments recommend waste discharge of about 200 ppm or less. A discharge higher than this is met with a heavy surcharge. Apart from the heavy penalties involved, the cost of treatment by the dairy or whey industry is usually very high in addition to valuable nutrients that are been wasted. Equipment and labor required to effectively treat whey and whey products are very costly. Jelen and Lemaguer (1976) indicated that the majority of unprocessed whey came from plants with prodution capacities of 9,000 kg or less. This shows the economic severity that will be faced by such small plants if they have to treat their wastes themselves.

C. Technical Problem

Beside the pollution problems, whey and whey products have some

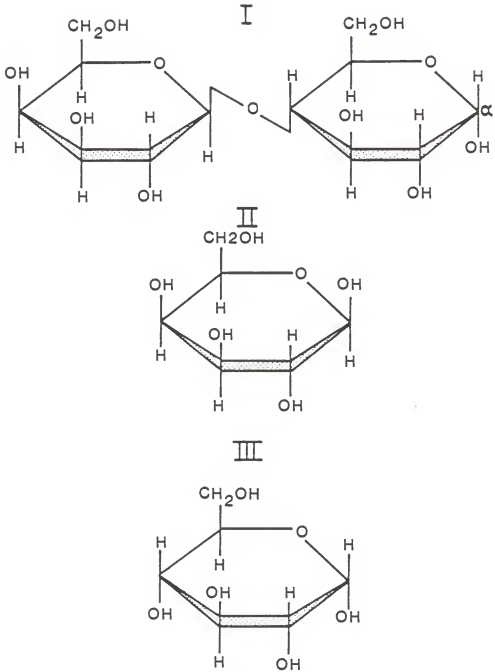


FIGURE 1 Structures of lactose (I), galactose (II), and glucose (III), The reducing residue is on the α-carbon atom of the glucose residue.

technical disadvantages which make their utilization quite unpopular. Lactose is the major solid in whey permeate. Its solubility and degree of sweetness is very low compared with other sugars (Table 2). An attempt to use high concentrations of it to compensate for the sweetness is marred with much less solubility and formation of crystals. Such crystal formation is considered a body defect in frozen dairy desserts a defect known as sandiness in ice cream.

D. Nutritional Problem

Lactose is the main carbohydrate found in milk. During digestion, it is hydrolyzed into glucose and galactose by the enzyme ß-galactosidase found in the intestinal mucosal cells (Alm, 1982). The fact that certain groups of the non-caucasian races are either deficient or have a low level of activity of the enzyme has been well documented (Alm, 1982; Richmond et al., 1981; Shukla, 1975; Zadow, 1984). The low level activity or lack of the enzyme in those individuals results in abnormality called lactose intolerance. About 70% of black Americans, 95% of certain Asian people and equally high percentages of the black Africans have this intolerance. The symptoms associated with this disorder include fermentative diarrhea, cramps, bloated feeling, belching and watery explosive diarrhea (Alm, 1982; Shukla, 1975; and Zadow, 1984). Figure 2 shows the frequency of lactose intolerance in percent of the population in some parts of the world (Alm, 1982).

IV. Whey Permeate Processing

Further treatment of whey permeate is necessary to maximize its utilization. Lactose is the principal component of permeate. Its hydrolysis using an immobilized enzyme system produces a more useful sugar that can

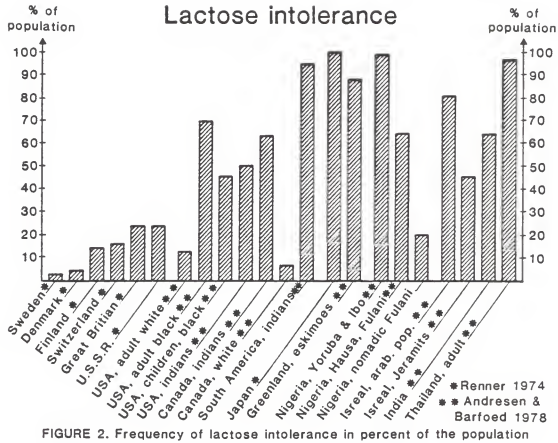


FIGURE 2. Frequency of lactose intolerance in percent of the population in some parts of the world. Adapted from Alm (1982).

be converted to a highly functional syrup. Such treatments will include demineralization, decolorization and concentration of the hydrolyzed whey permeate to syrups.

A. Obtaining Whey Permeate (UF Process)

Ultrafiltration (UF) is a process for separating solutes of different molecular weights according to the pore size of the membrane (Khorshid, 1974). The membrane pores are constructed in such a way as to allow micromolecules of certain solutes and that of solvents to pass through and be collected as permeate. Macromolecules, especially proteins, are retained on the membrane as retentate. The UF process is gaining popularity as a method for the concentration of milk prior to cheese making; however, its major application is in the fractionation process of whey to obtain whey protein concentrate (WPC) (Chiu and Kosikowski, 1985; Zadow, 1984). The UF process is expected to have more applications in the future as it has opened new possibilities for whey in the form of WPC and whey permeate. Its technology comes in different designs - spirals, hollow fiber, plate and frame, and tubes. Each has its own specific advantages, depending on the purpose of application (Petka and Mahon, 1984).

B. Demineralization

Demineralization is the process of removing the minerals and to reduce ash content of whey permeate. There are two techniques generally employed, electrodialysis and ion exchange. It has been shown that either of these techniques is capable of removing the mineral contents of permeate. However, the economics of such removal must be considered, especially at the commercial level.

An economic study by Ennis and Higgins (1982) showed that the use of ion exchange method for demineralization follows a linear relationship according to the extent of demineralization. Electrodialysis, on the other hand, is a self-attenuating process with a consequent disproportionate increase in costs at high level of demineralization. Kosikowski (1979) suggested that 60 to 70% demineralization by electrodialysis is the best for economics and elimination of salty flavor and mineral precipitation. In some cases, the demineralization not only removes the minerals, but also lowers the pH of the substrate allowing for more satisfactory hydrolysis (Zadow, 1984). Presence of high concentration of minerals can inactivate the ligand as well as aiding the formation of undesirable complexes.

C. Decolorization

This is a clarification process in which the color(s) in the hydrolyzed lactose (HL) is removed prior to concentration. Decolorization is generally done by using low concentration of activated carbon (charcoal). The Whey Products Institute has for some time now been urging the FDA to approve the use of other bleaching agents such as benzoyl peroxide (BPO) and hydrogen peroxide (H_2O_2) to bleach annatto-colored whey. This approval recently has been granted on July 30, 1986. (Federal Register 51, #146). The removal of color in HL makes it more adaptable for special uses such as in pharmaceutical and infant formula preparations where coloration is discouraged in the final products.

D. Concentration

Concentration of the HL is a means of obtaining hydrolyzed lactose syrup. It is carried out under vacuum with relatively low to medium heat. HL

can be concentrated to about 30 to 60% or more total solids, depending on the percent of hydrolysis. Shah and Nickerson (1978) indicated that greater than 70% TS aids crystal formation at 25°C. On the other hand, a 100% hydrolysis HL concentrated to 60% solids showed no crystal formation when stored at room temperature for one month (Chui and Kosikowski, 1985). The concentration process reduces the cost of transportation in addition to improving the shelf-life and sweetening properties of the hydrolyzed lactose syrup. A low to medium temperature range is prefered to prevent excessive burning and discoloration of the syrups (Wierzbicki and Kosikowski, 1973).

V. Immobilized Enzyme

Immobilized enzyme is defined as the localization or confinement of enzymes during a process. It is a technique that allows for the removal of the ligand from the substrate and the product for purpose of reuse. It fixes the enzyme so as to retain it in a continous process (Kilara and Shahani, 1979; Hultin, 1983). The technique for immobilizing enzyme was first used by Nelson and Griffin in 1916 in which they adsorbed invertase on charcoal and on alumina (Messing, 1975). The first inudstrial use of immobilized enzyme was in 1966 by the Tanaba Seiyaku Company Limited of Japan for the production of amino acids (Hultin, 1983; Carasik and Carroll, 1983). Immobilized enzyme technology offers some advantages over the usual soluble or free enzyme system. These include multiple or repetitive use, stability of the enzyme, long half-life, and easy separation of the enzyme from product(s) (Messing, 1975). There are a number of areas where this technique has been used in the food industry today. This includes the beer industry for the conversion of dextrin to dextrose using amyloglucosidase, in the production of high-dextrose syrup, conversion of whey to glucose and

galactose (using lactose) and most importantly, in the production of high-fructose corn syrup (HFCS) in the conversion of glucose to fructose by the enzyme glucose isomerase (Carasik and Carroll, 1983). An immobilized enzyme system is made up of several parts such as the matrix system, the reactor, the enzyme and the substrate.

A. Matrix System

The matrix system is also known as support or carrier. There are varieties of support systems that could be used and are generally classified into two broad groups - organic and inorganic matrices. Within these broad groups are also the classification into porous and nonporous support materials. Porous inorganic support such as controlled pore glass (CPG) and other glass particles colloidal silica and carbon are used primarily in covalent attachment (Messing, 1975). They are generally higher in surface area and have greater resistance to chemical and microbial attacks. Further, the inorganic supports are easy to handle, possess high mechanical strength, and are reusable (Filbert, 1975).

B. Type of Reactor

"There is no universal reactor system", says Messing (1975). The reactor of an immobilized enzyme must be tailored to the needs and applications of a given process. A good reactor must permit easy contact of the enzyme and the substrate and must be designed to allow easy cleaning to prevent microbial contamination and plugging, which will subsequently lead to the inactivation of the enzyme. Among basic reactors are batch, membrane, spunfibers, continous stirred tank, fluidized bed and the packed bed or plug flow reactors (Hultin, 1983).

C. Method of Immobilization

There are a number of techniques or methods available for immobilizing enzymes. A method chosen should be adequate and maximize the enzyme activity to the level desired. Kilara and Shahani (1979) mentioned that there is no best method as such; instead, the best method is the one that promotes maximum enzyme activity per unit of matrix.

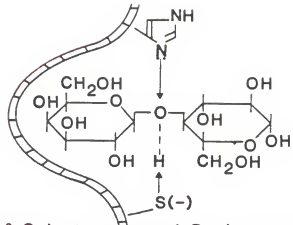
Common techniques or methods include entrapment within gel, adsorption, microencapsulation, ion-exchange, copolymerization, cross-linking, and covalent attachment (Kilara and Shahani, 1979).

The covalent method is the most widely used. It involves the reaction of the free carboxyl or the free amino groups of the enzyme to a suitable carrier to form covalent linkages (Weetall, 1975; Kilara and Shahani, 1979). The reaction is carried out in the presence of acylating or alkylating agent (e.g. aldehydes, isocyanates and diazonium salts).

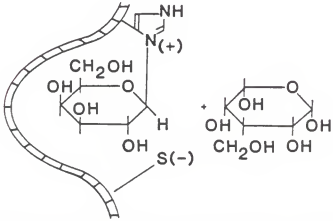
The use of aldehyde as an acylating agent involves the formation of a Schiff's base (Weetall, 1975, Kilara and Shahani, 1979). The coupling is formed between the amino carrier and the available amino group on the protein. The reaction is generally gentle and occurs at pH 7.0 followed by repeated washing steps to prevent crosslinking of the carrier with the protein. Plugging of the entire system will result should the enzyme be allowed to crosslink with the carrier (Weetall, 1975).

D. The Substrate

The activity of an enzyme is defined in terms of the amount of substrate converted per unit time per weight of enzyme (Carasik and Carroll, 1983). For many enzymes, the rate of catalysis varies with the substrate concentration. This chemical reaction classification is known as kinetics, it



A 4-O-β-Galactopyranosyl-D-glucopyranose molecule at the active site of β-galactosidase enzyme



β-Galactosidase-galactose complex + glucose

FIGURE 3 A. Proposed mechanism of lactose hydrolysis by beta-galactosidases.

is the study of reaction rate (Bohinski, 1983). Studies on enzymic reaction showed that at high substrate concentration, the enzyme is inhibited, thus leading to fall off in the initial reaction velocity of the enzyme (Fullbrook, 1983). In addition, substrate in IME system must be of the right size and viscosity to minimize steric problems (Hultin, 1983).

VI. Hydrolysis of Lactose

The hydrolysis of lactose by β-galactosidase is one of the major uses of immobilized enzyme technology in the food industry. The high content of lactose in whey permeate makes it a primary substrate for the immobilization system. There has been extensive publications on the mechanism (Figure 3A) of enzymic hydrolysis of lactose (Shukla, 1975; Kilara and Shahani, 1979; Richmond et al., 1981).

A. β-Galactosidase

Among the carbohydrase of particular interest is the β-galactosidase. The enzyme hydrolyzes the glycosidic bond between glucose and galactose of lactose (Kialra and Shahani, 1979). The enzyme β-galactosidase is also known as β-D-galactoside galactohydrolase. Other authors used the obsolete term lactase to describe the enzyme (Shukla, 1975). It is an enzyme capable of inducing the synthesis of certain oligosaccharides (Figure 3B) in a galactosyl transfer reaction (Richmond et al., 1981). The β-galactosyl transfer is a preferential reaction at the primary alcohol of D-glucose forming various di - and oligosaccharieds (Richmond et al., 1981). Among the major reactions of β-galactosidase is to catalyze the hydrolysis reaction of β-D-galactosides and L-arabinosides (Richmond et al., 1981). It is an enzyme well defined in literature and widely distributed in nature. β-Galactosidase can be found in

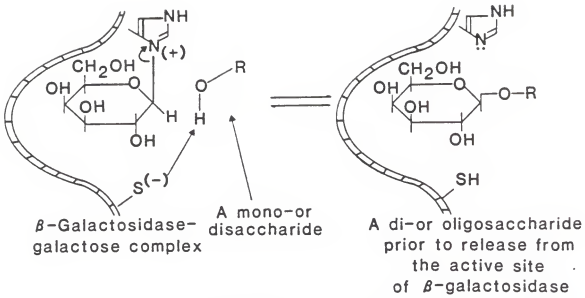


FIGURE 3 B. Proposed mechanism of galactosyl transfer reaction by beta-galactosidases.
Adapted from Shukla (1975)

plants, animal organs, yeasts, bacteria and fungi (Richmond et al., 1981; Shukla, 1975). Lactases are essentially similar in specificity, but vary in physicochemical, catalytic and kinetics properties depending on the source or origin (Richmond et al., 1981; Kilara and Shahani. 1979; Woychik and Wondolowski, 1974).

The enzyme has on its active site one sulfhydryl (-SH) and one imidazole group in a reaction that corresponds to S_N2 - like displacement mechanism. The number of active sites in the enzyme is temperature-dependent. This implies that at low temperatures not all the active sites are available (Richmond et al., 1981; Shukla, 1975; Wallenfels and Weil, 1972).

There are specific factors that affect the rate of hydrolysis of lactose by B-galactosidase. These include the time or duration of the reaction, temperature, concentration of substrate, and the pH of the system.

Figure 3A shows the proposed mechanism of lactose hydrolysis by beta-galactosidases. The hydrolyzed lactose yields glucose and galactose at equal molar concentration. The resultant monosaccharides are more functional than the unhydrolyzed lactose. Holsinger (1978) has reported that hydrolysis of lactose in whey or whey products results into a product that has different physical and chemical properties, which should be of great interest to the dairy manufacturer. She further mentioned that the hydrolyzed lactose now has a low lactose content, has little or no crystal formation, much sweeter sugars, and readily fermentable carbohydrates.

B. Oligosaccharides

Apart from catalyzing the hydrolysis of B-D-galactosides and -L arabinosides, B-galactosidase is also a synthetase, which effects the formation of certain oligasaccharides. It effects the transfer of the

galactose moiety of the lactose molecule to various accceptors such as monosaccharides, polysaccharides and alcohols in a process termed "transgalactosidation" (Aronson, 1952; Roberts and McFarren, 1953; Wierzbick and Kosikowski, 1973).

Only traces of oligosaccharides are formed in dilute lactose solutions, but as the lactose concentration increases, the percentages of oligosaccharides formed also increase (Nickerson, 1974). In addition to the substrate concentration, oligosaccharide formation is affected by the source of enzyme, the pH and temperature at which the reaction occurs (Shukla, 1975). Figure 3B shows the proposed mechanism for the oligosaccharide formation during the hydrolysis of lactose.

C. Determinaiton of Lactose

There are many methods available for the determination of lactose in a given product. Among these are Munson-Walker method (involving the formation of cuprous oxide), the chloramine T-method (back titration of liberated iodine) and polarimetry method (Zadow, 1984). In addition to the above methods, there are also enzymatic cryoscopic method (Zarb and Hourigan, 1979) and high pressure liquid chromatograhpy (HPLC) (Johnson, 1981; Jeon et al., 1984).

The determination of carbohydrates by the high performance liquid chromatography (HPLC) method is well investigated. The method is proven to be versatile, specific and can be performed with relative speed, adequate precision, and accurate results (Jeon et al., 1984). The very closely related compounds such as lactose, glucose and galactose are not easily distinguishable with the traditional carbohydrate assay methods (Woollard, 1983). However, chromatography methods such as HPLC and gas liquid

chromatography (GLC) have an advantage over the enzyme procedures because carbohydrate mixture can be assayed simultaneously (Woollard, 1983).

VII. Uses of Hydrolyzed Lactose

Only a small amount of whey permeate has been actually processed and used compared to its availability or production, due to problems associated with whey utilization. In an attempt to solve these problems, processes such as UF and immobilized enzyme are employed to process the lactose in permeate into a more usable end product (i.e., hydrolyzed lactose). Concentrating the HL makes it more versatile and can be used in baked goods and frozen dairy desserts.

A. Hydrolyzed Lactose in Baked Products

It has long been recognized that HL makes acceptable bread when used even in the absence of other bakery sugars (Pomeranz et al., 1962). Hofstrand et al. (1965) found that doughnuts containing lactose were significantly more compressible than those that did not have lactose. Lactases were added at various concentrations directly into the bread formulation to hydrolyze the lactose sugar present in the mixes. Their work showed that the doughnuts containing lactose were more tender and absorbed more fat than those without lactose.

Lactose is a reducing sugar with reactive aldehyde group which is important in the development of crust color. Lactose, in additon to dextrose, reacts with free amino groups of amino acids in what is known as Maillard reaction (Hugunin, 1980). The presence of lactose in bread and other baked goods has resulted in long shelf-life products and in more tender loaves.

Lactose is a good water binder, a property that aids emulsification property of shortening in dough mix.

Holmes and Lopez (1977) indicated that the replacement of 20 to 30% of sucrose with lactose is desirable in products that require low sweetness, limited solubility, strength, and hardness of crystal. The addition of HL to formulations under optimum conditions were found to have improved the appearance, flavor and aroma, volume and the shelf-life of the respective baked goods. Vetter (1984) reported the results of a series of experiments performed on the use of lactose in baked products. He found that lactose was very desirable in bakery products as its presence increased the loaf's specific volume and produced bread with good texture and acceptable grain.

B. Use in Ice Cream

Whey and whey products have been used more often in dairy products than in any other foods. Patel and Harper (1977) advocated the use of cottage-cheese whey as a replacement for milk solids-not-fat (MSNF) in frozen desserts as a natural acidulant. Rothwell (1979) called the HL a more functional ingredient that provides the ice cream manufacturer a real and more economical choice. The sugars, he says, "have two major advantages from the ice cream manufacturer's point of view." The first is that the residual lactose of the hydrolyzed sugars is not sufficient to cause crystallization; hence a major body defect is avoided. The second is that glucose and galactose are more soluble and much sweeter than unhydrolyzed lactose. Therefore, it is likely that less sugar than normal will be needed in the ice cream mix. In addition to all the advantages above, HL products are safer for individuals with lactose intolerance, since most of the lactose has been hydrolyzed.

Substitution with high levels of HL whey concentrate produced ice cream of good flavor, texture and smoothness (Lowenstein et al., 1975). They observed that hydrolyzed cottage cheese whey offers ease and economy of production and desirable overall qualities. An extensive project such as the one undertaken by Kirk Food called "Hydrolice" showed that HL makes better quality products. This work was designed to hydrolyze lactose in ice cream mix. The results showed that sweetness and freezing point of the resultant ice cream was greatly improved and at low temperature the ice cream was "found scoopable at a much lower temperature in the absence of glycerine" (Reissmann, 1982).

VII. Sensory Evaluation Methods

Sensory evaluation is a combination of the senses of taste, smell, touch and hearing when food is eaten (Larmond, 1977). It is an integral part of new product development and food quality control programs. The evaluation of the products may be done by one or more people, trained as well as untrained, depending on the product and the stage of development. There are three fundamental types of sensory tests: preference or acceptance tests, discriminatory tests, and descriptive tests (Larmond, 1977).

Preference or acceptance tests are affective tests. The personal feeling of the panelist directs his/her response. Discriminatory tests, on the other hand, determine if a difference exists between samples. Panelist should not be influenced by his own feeling or bias; rather he is encouraged to be objective and not subjective. The descriptive tests are used to determine the type and extent of differences in given samples.

The triangle test is the most popular of the difference tests (Brandt and Arnold, 1977). In this test, the panelist receives three coded samples,

two of which are exactly the same and one that is different. The panelist's task is to identify the odd sample (Larmond, 1977). When a substitution of one type or another is made in any product, the triangle test has been proven very easy to employ in determining the effect of such changes.

Comparison, hedonic-scale and ranking are all parts of preference tests (Larmond, 1977). Brandt and Arnold (1977) reported that the hedonic scale is the most widely used preference test. The term "hedonic" is defined as "having to do with pleasure" (Larmond, 1977). A nine-point hedonic scale is commonly used although, there are also a seven-point hedonic scale. Sample questionnaires are prepared and given to the panelists along with the product samples. They then rate the samples based on numerical values according to the scale chosen. The results of the rating are analyzed by statistical methods such as analysis of variance.

Multiple comparison test is another example of difference test. It is especially good to examine the effect of replacing or changing an ingredient, packaging technique and materials and changes in the process of storage. Several samples can be evaluated at a time with this test and it enables small differences between samples and control to be detected (Larmond, 1977).

MATERIALS AND METHODS

I. Immobilized Enzyme System

Immobilized enzyme (IME) technique was used in hydrolyzing the lactose in sweet whey permeates into its monosaccharide units. The IME unit used (Figure 4) was similar in design to the Corning's Lactose Hydrolysis Bench-Scale Unit. It consisted of four major parts: carrier, reactor, enzyme and substrate.

A. The components of the IME System

1. Carrier

The carrier consisted of silanized glass beads with particle diameter of 30/45 mesh and pore size of 375 Å (Corning Glass Works, Corning, NY).

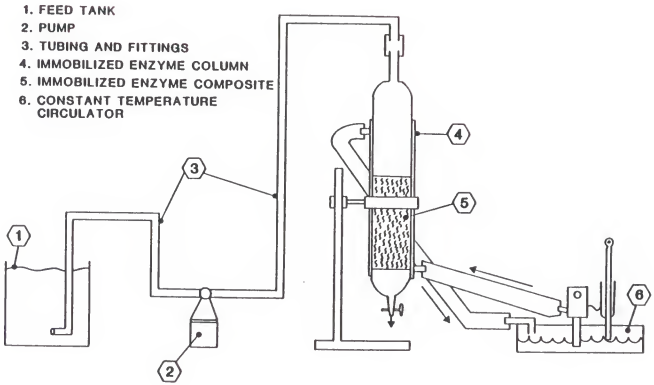
2. The Reactor System

The reactor consisted of a series of equipment that were connected together through fittings, tubes and joints. These include: Adjusta-chrom glass column, 25 X 600 mm (Ace Glass Inc., Vireland, NJ), Masterflex Pump Head Model 702420 with Masterflex Speed Controller (Cole-Parmer Instrument Co., Chicago, IL), and Fisher Model No. 80 Circulating Water Bath (Fisher Scienctific Co.).

3. The Enzyme

The enzyme was the Takamine Brand Fungal Lactase, a food grade Lactase (E.C.3.2.1.23 β-D-Galactoside galactohydrolase), from Aspergillus oryzae (Miles Laboratories, Inc., Elkhart IN). It has an activity of 30,000 F.C.C. LU/g at pH 4.5. One F.C.C. Lactase Unit (F.C.C.LU) is defined as the

FIGURE 4 - LACTOSE HYDROLYSIS BENCH SCALE UNIT



amount of the enzyme that will liberate one micromole of o-nitrophenol (ONPG) per min at pH 4.5 and 37°C.

4. The Substrate

Pure lactose solution (5%, w/v) was prepared from analytical grade lactose (Fisher Scientific Co.) and used for the preliminary studies. Whey permeate was obtained at the Kansas State University Dairy Processing Plant. It was derived from sweet Cheddar cheese whey that had been treated with an ABCOR-Sanitary Ultrafiltration Pilot System Spiral-Wound Modules (ABCOR Inc., Wilmington, MA) and pasteurized at 162°F for 18 sec. The pasteurized whey permeate was packed in a gallon size container and kept frozen until used.

B. Immobilization of Fungal Lactase to the Carrier

1. Carrier Preparation and Enzyme Attachment

The enzyme was attached to the matrix system in a method similar to that of Robinson et al. (1971). Two hundred fifty grams of silanized glass beads were weighed into a 500 ml beaker. Cold deionized water was added and the mixture was refrigerated at 4°C overnight. The water was then drained off and the glass beads resuspended in a cold solution of freshly prepared 1% (v/v) glutaraldehyde (GA) for 30 min. (GA=Grade II 25% Aqueous from SIGMA Chemical Co., St. Louis, MO). The GA was rinsed off a few times with cold deionized water and the glass beads suspended in cold sodium phosphate buffer solution (1M, pH 7.0) containing 0.01 M magnesium chloride. A 5 g weight of Fungal Lactase was then added slowly with continuous but gentle stirring and the slurry was kept undisturbed in refrigerator for 2 hr. The slurry was then washed several times with cold sodium phosphate buffer solution (1M, pH 7.0)

containing 0.1 M sodium chloride and stored as wet cake in a refrigerator until used.

II. Whey Permeate Preparation and Hydrolysis

Twelve gallons of frozen whey permeate were thawed and stirred in a sanitized stainless steel container. It was acidified to pH 5.0 with concentrated food grade HCl.

A. Hydrolysis

The IME system (Figure 4) containing the composite (mixture of enzyme and glass beads) was used to hydrolyze the whey permeate. The reactor column was maintained at the constant temperature of 50°C and the Masterflex Speed Controller and the flow adapter were used to adjust the flow rate. A void volume of 300 ml was allowed to elute the reactor column while at the same time the % total soluble solids of the permeate was monitored with a refractometer (used °Brix) to assure proper equilibrium of the reactor's affluent and effluent. The first batches of hydrolysates had a flow rate of 40 ml/min. However, to achieve the maximum hydrolysis (more than 95%) that would suit our research scheme, portions of the previously hydrolyzed permeates were recycled through the reactor for the second time at a flow rate of 20 ml/min. All the hydrolysates were promptly refrigerated until processed.

B. Preparation of Hydrolyzed Permeate into Variables

The hydrolyzed whey permeates (HWP) were prepared and divided into three batches based on their degree of hydrolysis. These were 50, 75, and 95% hydrolyzed lactose. Each of these three batches were further subdivided into

two main groups according to the type of mineral treatment, namely, demineralized and undemineralized. This brought the total variable batches of HWP to six. The processing of each batch was according to the flow chart in Figure 5.

1. Demineralization

The variables that were designated as "demineralized" were treated with ion exchange resins of Ultrapure (Mixed-Bed) Barnstead D 8902 Cartridges (Fisher Scientific Co). The demineralization was conducted at room temperature and at a flow rate of 40 ml/min.

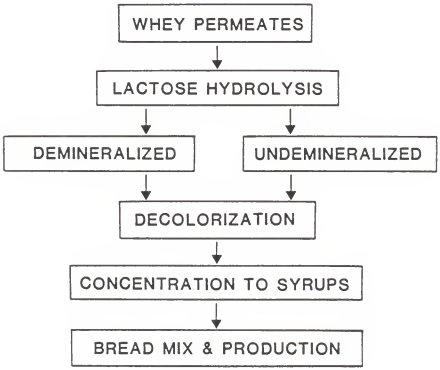
2. Decolorization

All the six batches of HWP were decolorized in a continuous flow mixed bed column containing silica gel with a particle diameter of 28/200 mesh (Davison Chemical, Baltimore, MD) and activated carbon of particle size 50/200 mesh (Fisher Scientific Co., Pittsburgh, PA) in a ratio of 1:2. Each batch was passed through the decolorizing column at a steady flow rate of 40 ml/min. The temperature of the column was maintained at 45°C.

3. Concentration

Decolorized HWP was concentrated in a 5L size round bottom boiling flask with an electric heating mantle. The flask was filled to about two-thirds of its volume with the HWP. All the units were operated at 50 to 65°C under vacuum. The degree of concentration was determined by Abbe Refractometer using the °Brix scale. Each batch of the hydrolyzed lactose syrups (HLS) was collected in a labelled flask, stoppered with cork and stored in a refrigerator

FIGURE: 5-HYDROLYZED LACTOSE SYRUP PRODUCTION AND USAGE IN BREAD MIX



until used in the bread mix formulation. To facilitate the concentration process, three evaporation units were operated simultaneously.

III. Bread Formulation and Production

The bread production steps are shown in Figure 6 while the equipment and their operating conditions are presented in Table 3. The HLS was used as variables in the bread formulation. Sucrose was used as control. Therefore, sweeteners were the only variables in the bread formulation. There were 21 batches of doughs for a total of 42 loaves of bread. Each batch of dough was coded and randomly selected for mixing and subsequent baking. Each of the seven variables was made in triplicate (3 x 2).

A. Ingredient Weighing and Mixing

The ingredients were weighed and/or measured on 700 g flour basis according to a typical white pan bread formulation used in KSU Baking Science 1 Laboratory (Table 4). The mixing process used was the straight-dough method in a Hobart A-200 mixer for a total of 6 min. The amount of water added to each batch of dough was adjusted to compensate for the water present in the HLS sweeteners as shown in Table 5. This compensation was based on the percent total solid contents in the HLS (Table 6).

B. Dough Fermentation

The mixed doughs were placed in lightly greased stainless steel fermentation bowls and set in the National Fermentation Cabinets (86°F, 86-90% RH). Each dough was punched after 2 hr and allowed to continue fermentation for additional ½ hr.

FIGURE: 6-STEPS INVOLVED IN BREAD PRODUCTION

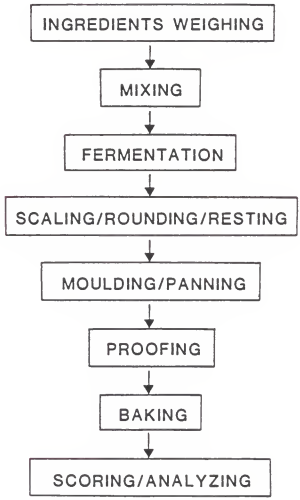


Table 3 - Baking equipment used during the hydrolyzed lactose syrups' breadmaking process.

Mixer: Hobart Model A-200
Fermentation: National Fermentation Cabinet (86°F; 90% RH)
Intermediate Proof: Drawer cabinet (for 20 min)
Moulder: Moline Moulder
Pan: 1 lb size baking pan
Proof Box: Anetsberger (105°F; 92% RH; Proofed to 1.5 cm above the pan)
Bake Oven: Reed Reel (425°F for 20 min)
Scale: Toledo Honest Scale
Volume measurement: Loaf Volume Meter

Table 4 - The general baking formula used for the hydrolyzed lactose syrup breads.

Ingredient	Bakers %[a]	% Total
Flour	100.0	56.4
Water (@ 60°F)	62.0	35.0
Yeast	1.0	0.6
Salt	2.0	1.1
Sugar	6.0	3.4
Shortening	3.0	1.7
NFDM	3.0	1.7
ARKADY[b]	0.25	0.1
		100.0

[a]Ingredients based on 100 parts flour (700 g)
[b]Yeast food
Method used was the straight dough, in white pan bread.

Table 5 - The amount of water added to each dough batch[a]

Dough	Sweetener	Water (ml)
1	95% DEM	392.0
2	50% UND	392.0
3	75% UND	400.0
4	CONTROL	434.0
5	95% UND	398.0
6	75% DEM	398.0
7	50% DEM	392.0

[a]The % TS in the syrups was taken into account to determine the amount of water added to each dough mix.
UND and DEM are undemineralized and demineralized hydrolyzed lactose syrup respectively.

Table 6 - pH and total solids of the hydrolyzed lactose syrups

% Lactose Hydrolyzed	pH	% TS[a]
50 UN	4.96	50
50 DE	4.88	50
75 UN	5.18	55
75 DE	5.72	54
95 UN	4.56	54
95 DE	6.11	50

[a]% TS was measured by refractometer.
UN and DE are undemineralized and demineralized HLS respectfully.

C. Dough Make-Up

The fermented doughs were then treated in the following steps:

1. Scaling

The dividing and the scaling are two process that complement each other. The fermented doughs were cut into 2 x 539 g pieces.

2. Rounding

The cut pieces of dough were rounded into a ball-like shape to form a smooth and continuous surface skin round the doughs.

3. Intermediate Proof

The rounded pieces of doughs were placed inside the bench drawers serving as the intermediate proof box for 20 min.

4. Moulding

At the end of the intermediate proofing the doughs were moulded by the Moline moulder into a loaf-like shape prior to panning.

5. Panning

The moulded doughs were panned in the lightly greased 1 lb size metal baking pans for final proofing.

6. Final Proof

The panned doughs were placed in the Anetsberger proof box (105°F, 92% RH) for the final proofing until a height of 1.5 cm above the pan was reached.

D. Bread Baking

The fully proofed doughs were baked in a Reed Reel oven at 425°F for 20 min. The finished loaves of bread were weighed and their volumes measured by Loaf Volume Meter immediately after baking. They were then cooled at room temperature for 1 hr after which the loaves were placed in polyethlene plastic bags and sealed by twist-tying.

IV. Analytical Methods

There were two phases to these studies; hence, two phases of analyses and/or evaluations were made. The first was the analysis related to whey permeates and the resultant hydrolyzed syrups. The second phase included the doughs and the loaves of bread. Whey permeate, hydrolyzed whey permeate, and the hydrolyzed lactose syrups were evaluated for pH, % TS, microbial standard plate count (SPC), mineral and nitrogen contents, and the degree of lactose hydrolysis. Analyses on doughs and loaves included: physical appearance, texture, pH, rate of gas production, bake-test, firmness, residual sugars and sensory preference tests. All the analyses on bread were performed in triplicate (3 x 2) with the exception of the residual sugars and preference tests which were performed in duplicate. Some of the data, primarily those obtained from the doughs and breads testing, were analyzed statistically with analysis of variance (ANOVA).

A. pH

All the pH measurements on the whey permeates, HWP and HLS were made using a Beckman pHI 43 pH Meter with ALTEX Thermo-Compensator ATC/Temperature Probe (Beckman Instrument Inc., Fullerton, CA). The pH

meter was calibrated on two-point standardization using certified standard buffer solution at pH 4.0 and 7.0 (Fisher Scientific Co) prior to each set of measurement. Test solutions were properly mixed using Flexa-mix Magnetic stirrer Model 16 (Fisher Scientific Co).

The pH's of the doughs were determined by a standardized method used in the KSU Baking Science 1 Laboratory. The pH meter used was Corning-Digital 110 Expanded Scale type.

B. % Total Solids

All analyses involving the total solids of whey permeates, HWP and HLS were determined with Refractometer using its Brix Scale (°B) (Bausch and Lomb Optical Co., Rochester, NY). Deionized water and standard sugar solution were used to calibrate the instrument. Test samples were analyzed at 20 ± 2°C.

C. Microbial Standard Plate Count

The microbial quality of the whey permeate and the hydrolyzed whey permeate were determined by the standard Methods for the Examination of Dairy Products (Richardson, 1985). Standard plate count agar was used in a pour plate with dilution factor (DF) of 10^0, 10^1 and 10^2. Plates were incubated at 32 ± 1°C for 48 ± 3 hr. The results were expressed as colony forming unit per ml (CFU/ml).

D. Minerals and Proteins

Whey permeate and the HLS were analyzed by the KSU Department of Animal Sciences and Industry Analytical service Laboratory for magnesium, sodium, potassium, calcium, phosphorus and chloride. Ash solutions were

prepared to be run through a Jarrell-Ash Atomic Absorption Instrument for the determination of Ca, Mg, Na and K. The spectrophotometric molybdovanadophosphate method (A.O.AC., 1984) was used for P determination. The titrimetric kit No. 830 (Sigma Chemical Co., St. Louis, MO) was used for determining Cl. Protein was analyzed by the Kjeldahl procedure (A.O.A.C., 1984).

E. % Lactose Hydrolyzed

The extent of hydrolysis of lactose in the whey permeate was determined by the high performance liquid chromatography (HPLC) procedure (Jeon and Mantha, 1985). The HPLC system consisted of a Beckman Model 100A pump, an Altex Scientific Model 210 injection valve with a 20µl loop and Model 156 refractive index (RI) detector, and a Fisher Recordall series 5000 recorder. The Amino Spheri-5 column (4.6 mm i.d.x 25 cm, Brownlee Labs., Santa Clara, CA) was operated at room temperature with a flow rate of 1.0 ml/min of 75% acetonitrile (HPLC grade, Fisher Scientific Co)/water mixture, which was deareated for 30 min with an ultrasonic machine. The guard column used was an Amino Spheri-5 polar phase AS-Gu cartridge (4.6 mm i.d. x 3.0 cm, Brownlee Labs., Santa Clara, CA). The chart speed was 0.5 cm/min for all analyses. One per cent standard carbohydrate solutions (lactose, glucose, and galactose) were prepared from analytical grade reagents in distilled water (w/V). Both the test solutions and the standards were prepared for injection using Sep-Pak C_{18} Cartridges (Waters Associates, Inc., Milford, MA) in a procedure described by Waters Associates (1981). The identification of the hydrolysates was made by the retention time of the test solution in comparison with the standards. Quantification was accomplished by using the ratios of the peak height of samples and the standards. Each analyses were performed in duplicate.

F. Fermentation

The rate of gas production (CO_2) in the dough was determined by a gasograph according to the procedure of Rubenthaler et al. (1980) except that 15 g of dough was used in the fermentation jars instead of the 10 g that they used in their studies. The process was monitored over a 2 hr period.

G. Specific Volume

The finished loaves of bread were weighed on the Toledo-Honest Scale and the volume determined by Loaf Volume Meter using the seed displacement principle. The ratio of the volume to the weight was used as the specific volume.

H. Firmness Test

The Voland-Stevens LFRA Texture Analyser was used to analyze the loaves for firmness. Each loaf of bread was sliced into nine equal slices (1 inch thick). The outer end slices and the innermost slice were discarded. The remaining six slices were used for the test. The instrument was operated under normal cycle and the plunger calibrated to penetrate a distance of 4 mm into the crumb at a speed of 2 mm/sec. Analyses were conducted on 1 and 3 days after baking.

I. Residual Sugars

The extraction method used for extracting sugars from the bread crumbs was similar to the one used by Kai (1985). A 20 g sample of crumb (from each batch of bread) was weighed into a 250 ml flask and 100 ml of 80% methanol (v/v) added. The alcoholic mixture was stirred vigorously for 3 min. The

mixture was then placed in Gyrotory Water Bath/Shaker Model G 76 (New Brunswick Scientific Co., Inc., Edison, NJ) and incubated at 50°C for 60 min with the medium speed shake. The mixture was filtered twice under vacuum through a Whatman No. 42 filter paper and the residues washed several times with a total of 20 ml 80% methanol. The filtrate was evaporated to about 15 ml in a 50°C water bath under vacuum. It was then poured carefully into centrifuge tubes (concentrating flask was rinsed with a small amount of deionized water which was also emptied into the tube) and centrifuged for 5 min at 10,000 rpm using Beckman Model J 21C centrifuge. The supernatant was carefully removed and made up to volume with deionized water in a 25 ml volumetric flask. The test solutions (supernatant) and carbohydrate standard solutions (1% W/V, fructose, glucose, galactose, maltose and lactose) were treated with Sep-Pak C_{18} as described under the % Lactose Hydrolyzed. The analytical procedures and operating conditions of the HPLC were similar to those used for the determination of % lactose hydrolyzed for whey permeate.

J. Bread Scoring

The finished breads were scored on specific characteristics. These were: crust and crumb color, texture, grain, symmetry, and break and shread. Two experienced bakers evaluated the bread and gave scores to each loaf based on the characteristics mentioned above. Comparisons were made between the hydrolyzed lactose syrups breads and the control breads. The average of the total scores was used to determine the best batch of breads.

K. In-House Sensory evaluation

To estimate a consumer preference on bread samples, an in-house sensory evaluation was conducted utilizing a total of 36 panelists. Most panelists were

KSU students and Office Staff members of Animal Sciences and Industry Department in Call Hall. They were given coded bread slices on a partitioned paper plates and were asked to score the samples on a hedonic scale of 7 (Larmond, 1977) utilizing evaluation sheets as shown in Appendix A. Average of their scores was used to determine which sample was most preferred.

L. Statistical Analysis

The analysis of variance (ANOVA) was used to analyze some of the data obtained from the dough and bread testings. The specific model used was designed by Dr. D. Johnson of the KSU Statistics Department. Two models were used: Model 1 was for some selective properties of the doughs and the the sensory analysis test data, while Model 2 was used for the firmness data.

Model 1. $Y_{ij} = \mu + R_i + T_j + E_{ij}$

Model 2. $Y_{ijk} = \mu + R_i T_j + P_{ij} + D_k + (TD)_{jk} + E_{ijk}$ (Split plot experimental design)

Where:

Y_{ij} = Response

R_i = Replicate effect

μ = Overall mean

T_j = Treatment effect

E_{ij} = Experimental error

D_k = Day effect

$(TD)_{jk}$ = Interaction between treatment and days

P_{ij} = Whole plot experimental design

E_{ijk} = Sub plot experimental error.

RESULTS AND DISCUSSION

I. Immobilized Enzyme System

A. IME System

The four major components of the IME system were integrated into the Laboratory Immobilized lactase bench scale unit (Figure 4). It represented a semi-closed continuous flow or packed bed column with multiple advantages over the batch-type system. The system was for all the intended purposes an efficient one. It effectively hydrolyzed the whey permeate without any indication of loss in ligand activity. It was easy to set up, required less operational area (operated on the laboratory bench top), and was relatively easy to operate. A problem encountered, however, was that of maintaining constant pressure in the reactor column. In a semi-closed system such as this, fluctuation in pressure is inevitable which can create a back-flow or remix of the substrate. The remix or overflow problem was solved by periodic shut down of the reactor pump thus preventing excessive build-up of pressure in the column.

B. Attachment of the Enzyme to the Glass Beads

The silanized glass beads was soaked overnight for maximum swelling of its particles and to increase its surface area for optimum ligand attachment. Maximum swelling of the glass beads was observed as it almost filled the 500 ml beaker. Being a porous inorganic matrix, it offers the advantages of strong mechanical strength that protects against microbial and chemical degradation in addition to having good flow properties (Messing, 1975; Kilara and Shahani, 1979). Silanization is an important step in the preparation of the support

materials for enzyme attachment. It is a process whereby the organo-functional reagent (such as silane) react with silanol or the oxide groups of the carrier. This process is also known as derivatization, and it provided the hydrocarbon arms on which the enzymes are attached (Messing, 1975; Kilara and Shahani, 1979).

The enzyme Fungal Lactase was covalently attached to the silanized glass beads using glutaraldehyde as the alkylating agent. Glutaraldehyde is a bifunctional reagent that promotes the cross-linking of the ligands to the matrix system. It selectively binds the protein groups that are not involved in the active site and also promotes the coupling of glass beads amine groups with the amine group of the protein. The reaction is believed to involve a Schiff's base formation and is gentle at neutral pH (Weetall, 1975; Kilara and Shahani, 1979). The IME technique used was perceived to be adequate based on the preliminary studies done with pure lactose solution (5% w/v). Utilizing the IME composite, a 100% hydrolysis of the pure lactose solution was obtained at a flow rate of 25 ml/min. The enzyme was also found stable after repeated useage for two days (8 hr/day); column was washed with 0.2% acetic acid solution (v/v) after each day use, which appeared to be effective for keeping the system free from microbial contamination.

II. Hydrolysis of Whey permeate and Hydrolyzed Lactose Syrup Preparations

1. Whey Permeate Hydrolysis

The initial pH of the whey permeate ranged from 6.26 to 6.40 and lactose content of 4.4%. Acidification of the permeate to pH 5.0 was necessary for its enzymic hydrolysis since the enzyme has an optimum activity at pH range of 4.5 to 5.0. Hydrolysis was achieved at 50°C instead of 55°C suggested by the

enzyme supplier. We operated at less than optimal temperature to give a margin of stability for the enzyme.

Substrates were pumped into the reactor column at a constant speed using the Masterflex speed controller and the column's flow adapter. A 300 ml void volume was eluted to bring the reactor to equilibrium with the substrate as well as to completely remove the buffer solution in which the composite was prepared and stored. The refractometer measurement of the column's effluent after the void volume was 4.4 °Brix as it was for whey permeate. The hydrolysates were then collected accordingly.

The flow rate for the first batches of hydrolysate was 40 ml/min. These batches achieved 80.6% lactose hydrolysis according to the HPLC analysis. The 80.6% hydrolysate was utilized for preparing 50 and 75% hydrolysis batches by mixing it in a proper ratio with unhydrolyzed whey permeate. To make 95% hydrolysis batches, a portion of the 80.6% hydrolyzed permeate was recycled through the reactor column for the second time. The flow rate for this batch was adjusted to 20 ml/min. Longer residence time was required for this batch due to the competitive inhibitory effect of galactose on the enzyme at the upper level of hydrolysis (Woychick et al., 1973; Kilara and Shahani, 1979). The twice hydrolyzed permeate showed a lactose hydrolysis of 95%.

2. Demineralization

The HWP batches designated as "demineralized" were treated with ion exchange cartridges to reduce their mineral contents. The purpose of this treatment was to evaluate the effect of minerals on the functionality of the HLS and the breads made from the syrups. The demineralizer cartridges used appeared to have affinity for some ions more than others (Tables 7A and 7B). As expected the major ions in the permeate were K and Cl, followed by Na, P,

Table 7A - Comparison of mineral content of demineralized and the undemineralized whey permeate[a]

	Content (ppm)					
	Mg	Na	K	Ca	P	Cl
UND	101.69	564.91	1605.91	342.54	359.89	1101.25
DEM	0.42	26.70	0.66	8.19	160.16	47.28

[a]Analysis was by A.O.A.C. (1984) method; samples were on wet basis, and all the data were in duplicate.

Table 7B - Comparison of mineral content of the demineralized and the undemineralized hydrolyzed lactose syrups[a]

	Content (ppm)					
	Mg	Na	K	Ca	P	Cl
UND	526.31	466.18	909.68	279.51	414.08	609.56
DEM	62.58	303.63	851.95	165.88	143.44	629.24

[a]Analysis was by A.O.A.C. (1984) method; samples were on wet basis, and all data were in duplicate (data did not accurately reflect the actual mineral content of the syrup samples especially the undemineralized HLS due to error in sample preparation for analysis).
UND and DEM are Undemineralized and Demineralized syrups, respectively.

Ca and Mg in that order (Table 7A). This order of magnitude was similar to the one reported by Hobman (1984) except that Ca and P were reversed. In addition to removing most of the ions, the demineralizer cartridges were also able to remove part of the greenish-yellow color of the permeate. However, the ability of the cartridges to remove color diminished as more permeate were passed through.

3. Decolorization

Decolorization of the HWP batches was done with the mixture of silica gel and activated carbon. Preliminary investigation indicated that using the activated carbon alone resulted in caking and very poor flow rate. This problem was resolved by mixing silica gel with the activated carbon in a 1:2 proportion. The mixture resulted in a better flow rate and at the same time removed the permeate's color very effectively. The decolorized HWP was as clear as water in appearance.

4. Concentration

The decolorized batches of HWP were concentrated to syrups under vacuum at 50 to 65°C. The temperature range used was desirable as it minimized the excessive burning and subsequent discoloration of the syrups. The undemineralized batches were characterized with foaming and formation of white precipitates (considered to be minerals and crystallized lactose) at the bottom of the heating flask. The undemineralized syrups were less viscous and not as visually appealing as the demineralized ones. In addition to the precipitated layer, they also had a slightly dark brown color and slightly salty taste. It was apparent from the time of concentration that the undemineralized syrups would pose some crystallization and stability problems during storage.

This problem may have led to the considerable differences in mineral contents between the undemineralized and demineralized syrups (Table 7B) as compared to the demineralized and undemineralized hydrolyzed whey permeate samples (Table 7A). It was beleived that the precipitates had some Cl ions entrapped in it, thus preventing homogenous sampling preparation and subsequently the lower level of Cl in the undemineralized HLS as opposed to the demineralized HLS. Therefore, data in Table 7B may not accurately reflect the actual mineral contents in the syrups.

5. Microbial Quality

The microbial analyses of the whey permeates (prior to acidification) indicated a low microbial content . The standard plate count on the permeates indicated 5.4×10^1 CFU/ml, whereas no colony was detected on the HWP. It is possible that the microorganisms initially present in the whey permeate were inhibited by the acidification and hydrolysis process at 50°C. It could also be that some of the materials used for the IME process (e.g. glutaraldehyde) have some antimicrobial properties that effectively inhibited their growth in the HWP.

III. Bread Formulation and Production.

A. Dough Development and Breadmaking

The hydrolyzed lactose syrups and the control sugar (sucrose) batches were coded and randomly used in the bread formulation mixes. All the ingredients were measured and/or weighed based on 700 g flour weight known as % flour basis or Bakers % (Table 4 and Figure 6). In a straight-dough method such as the one used, all the ingredients are added to the mixer bowl and

mixed in a single batch. This method offers the advantages of less processing time, labor, power and equipment needs. It also reduces fermentation losses (Pyler 1973; Ponte, 1978).

There appeared to be a slight difference in the handling property of doughs containing the HLS with respect to the degree of lactose hydrolysis and mineral contents of the syrups. The demineralized HLS dough were slightly moist and softer while the undemineralized HLS dough tended to be dry and somewhat bulky. The toughening appearance of the undemineralized HLS doughs may be due to the presence of ions such as Mg, P, and Ca in appreciable quantities in the syrups. The low level of hydrolysis (50%), however, appeared to have a drying effect on the dough. This is probably true since this batches contained more lactose which is a hygroscopic substance and a natural humectant. The syrups with the highest degree of hydrolysis (95%), on the other hand, had lower levels of lactose and appeared to favor slight softness of the dough.

B. Fermentation and Proofing

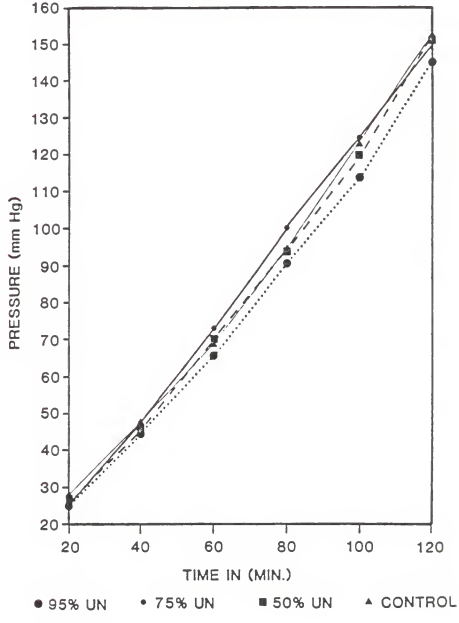
The panary fermentation or yeast fermentation is an important process in breadmaking. The yeast cells that are well dispersed in the dough system act on the fermentable sugars to produce carbon dioxide and alcohol as the major end products. However, not all sugars are fermented by the bakers yeast. Even those sugars that are fermentable are often done so preferentially and depend on a number of factors, such as the amount and the strains of yeast, amylolytic content and action of the flour and type and the amount of sugar present in the flour. The importance of sweetener in yeast dough goes beyond providing fermentable sugars for the yeast cells. It serves as flavoring and stabilizing agents, source of desirable nutrients, fermentation regulator and an important

factor in the crust color formation (Pyler, 1973; Pomeranz and Finney, 1975). The fermentation process in the HLS doughs were monitored with a gasograph. The gasograph is an instrument designed to measure and continously record the gas produced in fermenting doughs. Values of the gas produced are recorded as gasograph units (GU) which can be expressed as millimeters of mercury (mmHg) or cubic centimeters of gas by multiplying the GU by a factor of 7.3 or 2.38, respectively (Rubenthaler et al., 1980). There was a uniqueness to the pattern of fermentation activity of the HLS doughs, especially during the first 60 min and the later 60 min of the process (Figs. 7A & 7B). The undemineralized HLS doughs showed a slightly higher activity in the first 60 min of the fermentation process than the demineralized HLS doughs. It is possible that the presence of mineral ions (Table 7A) such as Ca and Cl in a much higher quantity in the undemineralized HLS than in the demineralied HLS triggered this reaction by stimulating the yeast cells to act faster since the said ions are commonly used in the yeast food preparation as stimulators. The trend of the fermentation pattern was reversed by the early part of the second-hour when the demineralized HLS doughs were more active than the undemineralized HLS doughs (Figures 7A and 7B). It is important to emphasize here that even though there may appear to be a difference in the activity rate among the respective HLS doughs, the statistical analysis of the fermentation process relative to each dough showed no significant difference.

All yeast-leavened products exhibit pH and total titratable acidity (TA) optima that can be related to the degree of fermentation which can then be related to optimum finished product characteristics (Mathason, 1977). In a freshly mixed dough, the pH is around 6.0 which decreases to about 5.0 during the fermentation. The changes are due to the production of carbonic acid as well as other minute organic acids in the dough system (Hoseney, 1986). The pH

FIGURE 7A

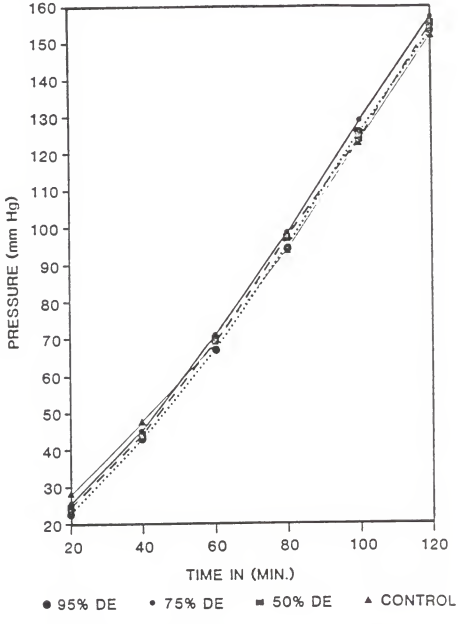
FERMENTATION OF UNDEMINERALIZED HLS IN DOUGHS



Plot was based on the average data of 3 x 2 doughs per batch of sweetener over fermentation period of 2 hr.

FIGURE 7B

FERMENTATION OF DEMINERALIZED HLS IN DOUGHS



Plot was based on the average data of 3 x 2 doughs per batch of sweetener over fermentation period of 2 hr.

of the HLS doughs after fermentation ranged from 5.35 to 5.64 (Table 8), and they were not statistically different.

The final proof is the last stage in the make-up process. It provides the dough with the relaxation and recovery time from the physcial stress and abuse of the previous stages. It also enables the dough to regain its strength, be aerated and acquire mellowness and extensibility (Pyler, 1973). The proof time is defined as the time in minutes that it takes the dough in the pan to rise to a given standard height above the pan. It varies from bread to types of process used. Most breads, however, are said to proof in the range of 55 to 65 min with 60 min being the optimum (Ponte, 1978). The HLS doughs proofed within 60.67 to 65.00 min (Table 8). The proof time was the time it took the dough to rise to the height 1.5 cm above the pan. The 50% HLS doughs took slightly longer to proof on the average, perhaps because these batches were limited in the yeast fermentables (it has low concentration of glucose and high concentration of lactose).

C. Bread Baking and Specific Volume

The last and the most crucial step in the breadmaking process is baking. During this process, the heat action of the oven transforms the unpalatable dough into a light, porous, readily digestible and flavorful product (Pyler, 1973). The fully proofed doughs were baked in the oven at 425°F for 20 min. The finished loaves were immediately weighed and their volumes measured with the Loaf Volume Meter. They were then cooled for 1 hr at room temperature and wrapped in 1 lb size bread plastic bags and saved for subsequent analyses.

The specific volume of the finished breads were determined using the ratio of the loaf's volume to its weight. It can be used as a measure of breadmaking process efficiency in conjunction with the bread's grain. A poorly

Table 8 - Selected data on hydrolyzed lactose syrup and control doughs and breads[a]

Sweetener	Fermentation[b] (mm Hg)	Proof (min)	pH	Sp.Vol (cc/g)	Avg.Score[c]
50% UND	151.60	65.00	5.44	5.50	30.0
50% DEM	155.00	64.67	5.35	5.59	30.0
75% UND	149.63	60.67	5.64	5.75	30.3
75% DEM	156.27	61.33	5.39	6.03	30.7
95% UND	129.47	62.67	5.43	5.52	29.3
95% DEM	153.53	60.67	5.39	5.41	27.0
SUCROSE	152.33	62.67	5.32	5.93	28.7

[a]Based on the average data of 3 x 2 doughs and/or breads.
[b]mm Hg was measured by Gasograph over 2hr period.
[c]Average score based on the loaf's crust and crumb color, texture, grain, volume and break and shread (scoring was by two experienced bakers).
UND and DEM are undemineralized and demineralized syrup respectfully.

mixed dough or improperly fermented dough would yield a poor quality loaf. Likewise, dough with unbalanced amount of ingredients would result in less than optimum quality bread. The specific volume of the experimental loaves varied from 5.41 to 6.03 cc/g. There was no significant difference in the specific volume of all loaves. However, the demineralized 75% HLS breads appeared to have slightly higher specific volume than the other batches of bread, followed by the control breads (Table 8). Since the demineralized 75% HLS doughs showed the most active fermentation activity overall, it was not surprising that it had the highest specific volume.

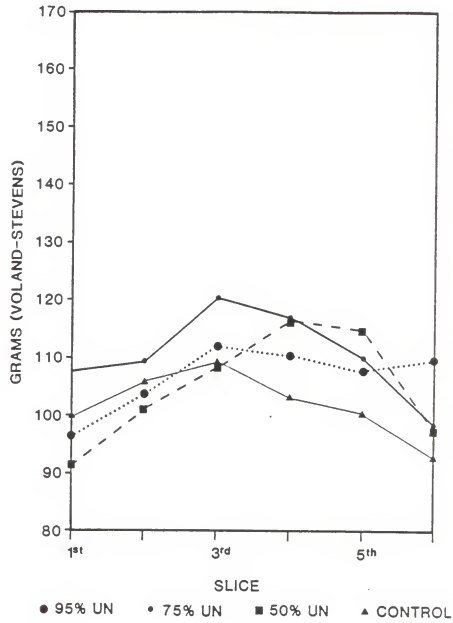
D. Bread Scores and Physical Characteristics

Bread scoring is fundamental to the overall evaluation of the breadmaking process. Breads are often scored to determine their physical characteristics. Among the characteristics that are generally used for this evaluation are: volume, color and nature of crust, texture, color of crumb, grain, uniformity of bake and symmetry of form (Pyler, 1973). Two experienced bakers scored the experimental loaves based on the physical characteristics mentioned above. Points were assigned to each loaf in each category and the average of the total score in each category was used to determine the overall best bread (Table 8). The control loaves were used as the standard with which the HLS breads were compared. The control breads and the demineralized 75% HLS breads had the best crust color. The 50% HLS breads regardless of their mineral treatments had darker crust color. An explanation for this could be due to the presence of the reducing lactose sugar in higher concentration in this batch (than in any other HLS batches) which readily enters into non-enzymatic browning reactions with amino groups. The presence of lactose in bread have been shown to play a major role in the crust color formation (Holmes and

Lopez, 1977; Shogren et al., 1979). On the crumb color, the undemineralized HLS breads received the highest score over the demineralized HLS and the control breads. It could be that the presence of the minerals have some desirable influence on the crumb. Both the demineralized and the undemineralized HLS breads appeared to have similar grain and textural characteristics. They were both scored equally and slightly higher than the control loaves (especially the 50 and 75% batches).

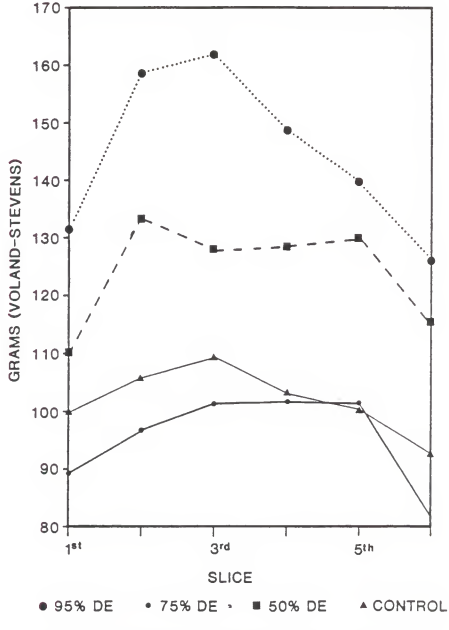
There are many concurrent changes that take place in bread during storage. All these changes are part of a complex process that is generally called staling. As bread ages, its crumbs begin to firm, followed by an increase in the degree of crumb-texture harshness, a loss of crust crispness and the disappearance of "fresh bread" flavor (Kulp and Ponte, 1981). Firmness is believed to be temperature-dependent among many other things (Pyler, 1973) and is an important sensory attribute of bread (Redlinger et al., 1985). There was a typical pattern to the firmness of the experimental loaves. All the loaves showed the middle slices to be the firmest. This was in agreement with the findings of researchers such as Ponte et al. (1962); and McDermott (1974). In addition, the combined effect of hydrolysis and demineralization of the HLS seemed to play a role in the crumb firmness. At both the 1-day and 3-day tests, the demineralized 75% HLS loaves showed the least firmness. However, the undemineralized 75% HLS breads were the firmest at both 1-day and 3-day testing periods. Undemineralization tended to improve crumb softness especially at 95% hydrolysis, but not as good as the demineralized 75% HLS. The control loaves were second best to the demineralized 75% HLS loaves over the testing periods (Figures 8A to 9B). The explanation for the least amount of firming in the demineralized 75% HLS loaves could be associated with the presence of moderate amount of lactose and minerals in this batch of syrups. Lactose in

FIGURE 8A FIRMNESS MEASUREMENT IN UNDEMINERALIZED HLS BREAD (ONE DAY OLD)



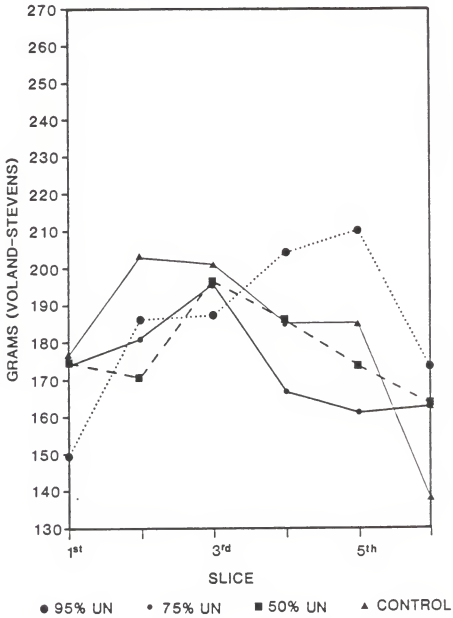
Plot was based on the average data of 3 x 2 loaves of bread per batch of sweetener(6 slices/loaf).

FIGURE 8B FIRMNESS MEASUREMENT IN DEMINERALIZED HLS BREAD (ONE DAY OLD)



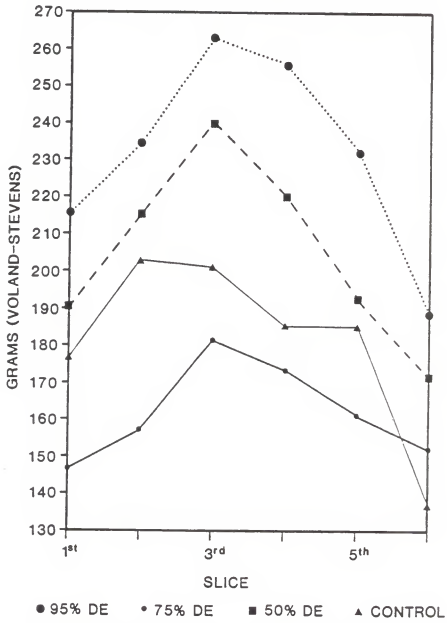
Plot was based on the average data of 3 x 2 loaves of bread per batch of sweetener (6 slices/loaf).

FIGURE 9A FIRMNESS MEASUREMENT IN UNDEMINERALIZED HLS BREAD (THREE DAY OLD)



Plot was based on the average data of 3 x 2 loaves of bread per batch of sweetener (6 slices/loaf).

FIGURE 9B FIRMNESS MEASUREMENT IN DEMINERALIZED HLS BREAD (THREE DAY OLD)



Plot was based on the average data of 3 x 2 loaves of bread per batch of sweetener (6 slices/loaf).

bread formula has been shown to significantly improve the bread's tenderness and shelf-life (Homes and Lopez, 1977).

E. Residual sugar content

Residual sugars are the sugars that remain in the dough after the yeast action has ceased. Sugars are fermented at different rate in the dough which will also mean that the residual sugar content of the crumb will vary. This variation will depend on the type of process used, type and the amount of initial sugars in the dough, the amount of alpha-amylase available in the dough and the baking condition (Tang et al., 1972; Ponte, 1978). The identification of the residual sugars were based on the HPLC retention time of the sample solutions in comparison with the standard solutions. The quantification was based on the ratios of the peak height of samples and the standards. The HPLC chromatograms for the residual sugar analyses are shown in Figures 10 through 13. Since residual sugars contribute to the sweetness of the finished bread based on their relative sweetness, one would expect the loaf with more residual fructose and maltose to be sweeter than the one having higher residual galactose and lactose at similar concentration. The control had higher residual fructose while the HLS breads showed traces of fructose but relatively high maltose, galactose and lactose (Table 9). Thus the control bread should be sweeter; however, this did not appear to be a factor according to the sensory evaluation. All the bread samples showed an unknown compound eluting through the HPLC column (Figs. 10-13) with the exception of the demineralized 75% HLS samples (Fig. 12E) that showed two unknown peaks. The single unknown peak was also observed by Kai (1985). It was not clear why the demineralized 75% HLS bread samples have two unknown peaks. The second peak may have been the compound that sets this batch of HLS breads apart from the rest. It is

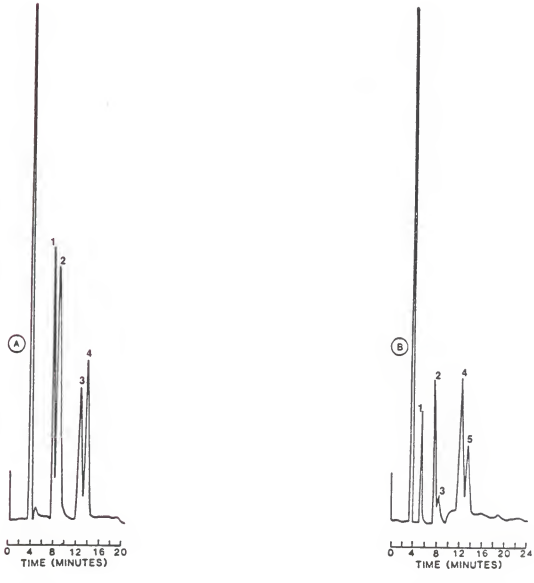


FIGURE 10 - High performance liquid chromatography chromatograms of standard solution and control bread extract at 1.0 ml/min flow rate.

(A) 1% standard sugar solution: 1)fructose, 2)glucose/galactose, 3)maltose and 4)lactose.
(B) Extracted from control bread, 20 g crumb in 80% methanol (v/v) : 1)unknown, 2)fructose, 3)glucose/galactose, 4)maltose and 5) lactose.

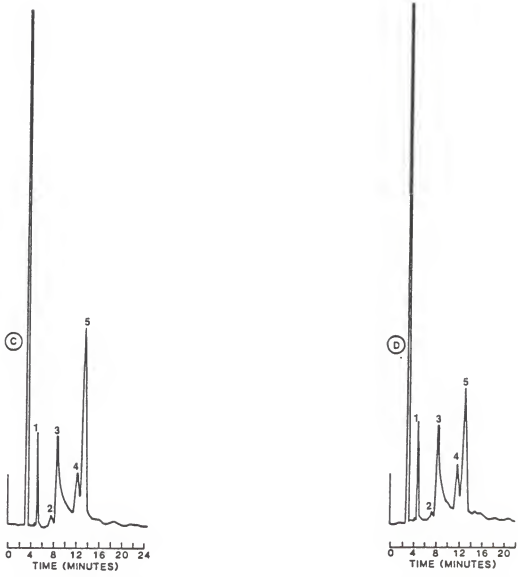


FIGURE 11 - High performance liquid chromatography chromatograms of sugar extract from 20 g crumb in 80% methanol (v/v) at 1.0 ml/min flow rate.

(C) Demineralized 50% HLS bread: 1)unknown, 2)fructose, 3) glucose/galactose, 4)maltose and 5)lactose.
(D) Undemineralized 75% HLS bread; 1)unknown, 2)fructose, 3) glucose/galactose, 4)maltose and 5)lactose.

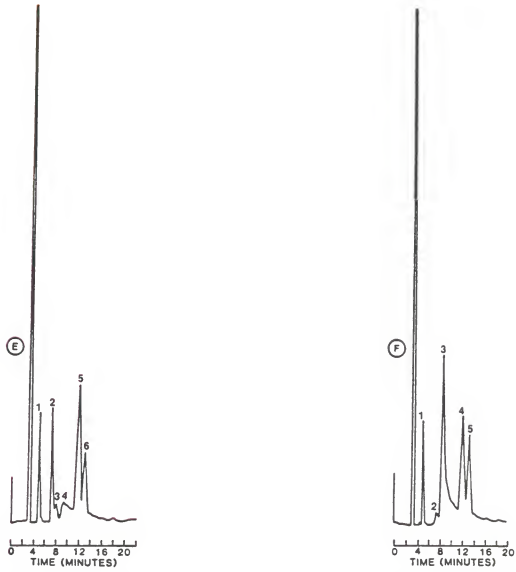


FIGURE 12 - High performance liquid chromatography chromatograms of sugar extract from 20 g crumb in 80% methanol (v/v) at 1.0 ml/min flow rate.

(E) Demineralized 75% HLS bread: 1 and 2)unknowns, 3)fructose, 4)glucose/galactose, 5)maltose and 6)lactose.
(F) Undemineralized 95% HLS bread: 1)unknown, 2)fructose, 3) glucose/galactose, 4)maltose and 5)lactose.

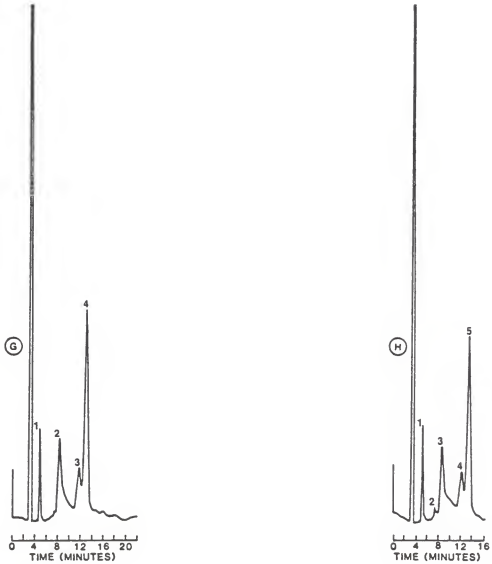


FIGURE 13 - High performance liquid chromatography chromatograms of sugar extract from 20 g crumb in 80% methanol (v/v) at 1.0 ml/min flow rate.

(G) Demineralized 95% HLS bread: 1)unknown, 2)glucose/galactose, 3)maltose and 4)lactose.
(H) Demineralized 50% HLS bread: 1)unknown, 2)fructose, 3)glucose/galactose, 4)maltose and 5)lactose.

Table 9 - Residual sugars in the hydrolyzed lactose syrup and control bread crumbs[a]

Sweetener	% Sugar Concentration in bread			
	Fructose	Gluc/Gala[b]	Maltose	Lactose
UN 50%	0.05	0.41	0.32	1.47
DE 50	0.04	0.34	0.36	1.40
UN 75%	0.03	0.47	0.44	0.99
DE 75	0.03	0.08	1.20	0.49
UN 95%	0.03	0.81	0.92	0.64
DE 95	*	0.40	0.41	1.60
CONTROL	0.66	0.07	1.28	0.55

[a]Analysis was by HPLC method using the retention time and the peak height ratio of samples and the standards. 20 g of crumb was dissolved in 100 ml of 80% methanol (v/v) for the sugar extraction.
[b]Glucose and galactose were eluted as single peak under the column condition used.
*Not detected.
UN and DE imply the undemineralized and the demineralized syrups, respectively, and the % is for the degree of lactose hydrolyzed.

worthy to note that the demineralized 75% HLS breads possess a unique ability and exceptional performance and quality above all the other HLS breads in almost all of the tests performed. It is also noted that this batch of bread showed a significantly higher residual maltose similar to that of the control breads (Table 9). The higher residual lactose found in the 50% HLS batch explained why the crust color of the loaves from this batch was darker than the others.

F. In-house Sensory Evaluation.

The results of the in-house sensory evaluation indicated how much of likeness or dislike the panel had for the HLS and the control breads. These results are summarized in Table 10. A total of thirty-six panelists evaluated the coded samples on a hedonic scale of 7-points (Appendix A). The demineralized 50% HLS bread had a total of 205 points being the highest of all the samples. However, this included only 16.7% of the judges prefering it as "like very much" and 2.8% as "dislike slightly." In comparison the control bread had a total score of 196 points with 19.4% of the panelists indicating it as "like very much" and 8.3% "dislike slightly." The demineralized 75% HLS samples, on the other hand, had a total point of 193 but was "like very much" by 22.2% of the panel and only 2.8% "dislike moderatley." Most of the panelists indicated that the HLS breads in general were more flavorful and possessed good grain and texture than the control breads. Perhaps, these results could be considered as an indication of potential acceptance for the HLS breads by prospective consumers. There was no indication of an outright rejection of any of the HLS breads.

The results of the proximate analysis on the loaves is shown in Table 11. This analysis shows the undemineralized loaves as having higher ash content

Table 10 - Summary of the in-house evaluation on the hydrolyzed lactose syrup and the control breads[a].

BREAD I.D[b]	TOTAL POINT	AVE. SCORE	RANGE
UN 50%	193	5.4	3-7
DE 50	205	5.7	3-7
UN 75%	181	5.0	2-7
DE 75	193	5.4	3-7
UN 95%	187	5.2	2-7
DE 95	195	5.4	3-7
CONTROL	196	5.4	3-7

[a]The test was conducted with a total of 36 panels. Scoring was on hedonic scale of 7; 1= dislike very much and 7= like very much

[b]Identification of syrup batches from which the bread was made, with UN and DE indicating undemineralized and demineralized syrups, respectively.

than the demineralized HLS loaves. Thus it could be said that the demineralization process was somewhat effective.

Since the demineralized 75% HLS breads showed the most promising and desirable results, we would recommend further investigation of this product to optimize its potentials. The use of HLS in bread formulation according to our understanding is a relatively new area, and yet a very promising and challenging one. Further work is therefore needed to highlight some of the findings of this research.

Table 11 - Proximate analysis of the hydrolyzed lactose syrup and the control breads[a].

	%					
SWEETENER	DM	ASH	CP	FAT	CF	NFE
50% UND	62.38	3.11	12.59	2.24	0.49	81.55
50% DEM	66.78	2.62	12.78	2.29	0.76	81.53
75% UND	63.26	3.17	12.66	2.27	0.58	81.30
75% DEM	64.15	2.22	12.83	2.14	1.15	81.63
95% UND	63.51	3.20	12.20	2.43	1.37	80.77
95% DEM	63.64	2.34	11.28	2.13	1.39	82.83
SUCROSE	65.15	2.31	12.70	1.50	0.38	83.09

[a]Analysis was by A.O.A.C. (1984) method and all data were in duplicate. DM - dry matter; CP - crude protein; CF - crude fiber; and NFE - nitrogen free extract; UND - undemineralized and DEM - demineralized.

CONCLUSION

The following conclusions can be drawn based on the insights gained from this investigation.

1. Quality food grade syrups can be made from hydrolyzed whey permeates.
2. The functionality of the syrups can be optimized by relatively reducing its mineral contents.
3. Decolorization of the syrups is recommended to remove the whey-tainted flavor and the greenish-yellow color of the whey.
4. Either too low or too high percentage in lactose hydrolysis of the whey permeate (50 and 95%) appeared to have no significant functional advantage for the syrups and its usage.
5. The hydrolyzed lactose syrups can be used to substitute sucrose in the white pan bread formulation.
6. The extreme cases of lactose hydrolysis and presence of large quantity of mineral ions in the syrups seemed to have some adverse effect on the characteristics of the doughs.
7. The demineralized 75% hydrolyzed lactose syrups appeared to have the best functional qualities for use in bread mix formulation.
8. There seemed to be enough fermentable sugars and relative amounts of minerals in the demineralized 75% HLS to effectively support the vital fermentation and chemical reactions of the doughs and subsequently yielding quality bread

9. Breads from the demineralized 75% HLS syrup were comparable to the control breads and in some instances were ranked to be of better quality.
10. The demineralized 75% HLS breads were the most desirable, consistent, and the best in almost all the categories investigated.
11. The results of the sensory evaluation of the HLS breads showed a satisfactory level of acceptance for the HLS breads.

REFERENCES

Alm, L. 1982. Effect of fermentation on lactose, glucose, and galactose content in milk and suitability of fermented milk for lactose intolerant individuals. J. Dairy Sci. 65:346.

A.O.A.C. 1984. "Official Methods of Analysis". 14th ed. Association of Official and Analytical Chemists, Washington, DC.

Aronson, M. 1952. Transgalactosidation during lactose hydrolysis. Arch. Biophys. 39:370.

Bohinski, R.C. 1983. Modern Concepts in Biochemistry 8th ed. Allyn and Bacon Inc., Boston, MA. p. 132.

Carasik, W and Caroll, J.O. 1983. Development of immobilized enzymes for production of high fructose corn syrup. Food Technol. 37(10):85.

Chandan, R.C., Uebersax, M.A.,and Saylock, M.J. 1982. Utilization of cheese whey permeates in canned beans and plums. J. Food Sci. 47:1649.

Chiu, C.P. and Kosikowski, F.V. 1985. Hydrolyzed lactose syrup from concentrated whey permeates. J. Dairy Sci. 68:16.

Elmer, R.A. and Clark, W.S.Jr. 1982. Whey of doing it. Modern Brewery Age. Whey Products Institute, Chicago, Il. (December, 20).

Ennis, B.M. and Higgins, J.J. 1982. Demineralization of deproteinated sweet whey - an economic study. N.Z.J. Dairy Sci. and Technol. 17:27.

Filbert, A.M. 1975. Controlled-pore glasses for enzyme immobilization. In "Immobilized Enzymes for Industrial Reactors". Ed. Messing, R.A. Academic Press, New York. p 39.

Fullbrook, P.D. 1983. Practical applied kinetics in industrial enzymology. In "The Application of Enzymes in Industry". Eds. Godfrey, T. and Reichelt, J. Macmillan Pub. Ltd., England.

Giec, A. and Kosikowski, F.V. 1983. Low temperature lactose hydrolysis of concentrated whey permeates. J. Dairy Sci. 66:396.

Harper, W.J., Blaisdell, J.L, and Grosshopf, J. 1971. Dairy food plant wastes and waste treatment practices. National Technical Information Service #PB-231, 372. U.S. Dept. of Commerce, Springfield, VA.

Hausser, A.G. 1984. Lactose hydrolysis of whey permeate using a continous flow-through immobilized enzyme system. Proceedings Whey Products Conference. Agricultural Research Service U.S. Dept. of Agriculture.

Hobman, P.G. 1984. Review of process and products for utilization of lactose in deproteinated milk serum. J.Dairy Sci. 67:2630.

Hofstrand, J.T., Zaehringer, M.V. and Hibbs, R.A. 1965. Functional properties of two components of cheddar cheese whey in bakery products. 1. Cake

doughnuts. Cereal Sci. Today. 10:213.

Holmes, D.G. and Lopez, J. 1977. Lactose in bakery products - an update. Bakers Digest. 51(1):21.

Holsinger, V.H. 1978. Applications of lactose modified milk and whey. Food Technol. 32(3):35.

Horton, B.S. 1982. Permeate update - current processing technology. Proceeding, Whey Products Conference. Agricultural Research Service U.S. Dept. of Agriculture.

Hoseney, C.R. 1986. Principles of Cereal Science and Technology. A.A.C.C., Inc., St. Paul, MN. pp. 203-222.

Hugunin, A.G. 1980. Whey - an opportunity for the baking industry. Bakers Digest. 54(4):8.

Hultin, H.O. 1983. Current and potential uses of immobilized enzymes. Food Technol. 37(10):66.

Jelen, P. and Lemaguer, M. 1976. Feasibility evaluation of cheese whey processing in small plants. J. Dairy Sci. 59:1347.

Jeon, I.J., Galitzer, S.J. and Hennessy, K.J. 1984. Rapid determination of lactose and its hydrolyzates in whey and whey permeates by high performance liquid chromatography. J. Dairy Sci. 67:884.

Jeon, I.J. and Mantha, V.R. 1985. High performance liquid chromatography analysis of oligosaccharides formed during ß-galactosidase action on lactose. J. Dairy Sci. 68:581.

Kai, T. 1985. Comparison of residual sugar and firming characteristics of white pan breads made by sponge dough and short-time dough processes. M.S. Thesis, Kansas State University, Manhattan, KS.

Khorshid, M.A. 1974. Studies on the permeate from the ultrafiltration of whey. Aust. J. Dairy Technol. 29:37.

Kilara, A. and Shahani, K.M. 1979. The use of immobilized enzymes in food industry: a review. CRC. Crit. Rev. Food Sci. Nutr. 12:161.

Kosikowski, F.V. 1978. Cheese and Fermented Foods. 2nd ed. F.V. Kosikowski & Associates, Brooktondale, NY. p. 446.

Kosikowski, F.V. 1979. Our industry today: whey utilization and whey products. J. Dairy Sci. 62:1149.

Kulp, K. and Ponte, J.G, Jr. 1981. Staling of white pan bread: fundamental causes. CRC Crit. Rev. Food Sci. Nutr. 15:1.

Larmond, E. 1977. Laboratory Methods for Sensory Evaluation of Food. Publication #1637. Research Branch-Canada Dept. of Agriculture, Ottawa, Ont., Canada. p. 19.

Loewenstein, M., Reddy, M.B., White, C.H., Speck, S.J., and Lunsford, T.A. 1975. Using cottage cheese whey fractions or their derivatives in ice cream. Food Prod. Dev. 9(9):91.

Luksas, A.J. 1984. Use profile for whey products obtained from ultrafiltration-treated milk. Proceedings Whey Products Conference, Agricultural Research Service, U.S. Dept. of Agriculture.

Mathason, I.J. 1977. pH and total titratable acidity for process control and formulation. Bakers Digest. 51(1):52.

Mathason, I.J. 1978. pH and T.T.A. determination for fermentation control. Bakers Digest. 52(3):10.

McDermott, E.E. 1974. Measurement of the stickiness and other physical properties of bread crumb. J.Food Technol. 9:185.

Messing, R.A. 1975. Immobilized Enzymes for Industrial Reactors. Ed. Academic Press, NY. pp. 1, 63.

Nickerson, T.A. 1974. In "Fundamentals of Dairy Chemistry". 2nd ed. Eds. Webb, B.H., Johnson, A.H., and Alford, J.A. AVI Publishing Co., Inc., Westport, Ct. p. 273.

Patel, A.R. and Harper, W.J. 1977. Utilization of acid whey in ice cream. Dairy & Ice Cream Field 160(6):70A.

Pazur, J.H. 1970. Oligosaccharides. In "The Carbohydrates: Chemistry and Biochemistry. Ward Pigman et al. Eds. Academic Press, New York, NY. p. 69.

Petka, T.E., and Mahon, A.P. 1984. Producing value-added whey products using membrane technology. Proceedings, Whey Products Conference, Agricultural Research Service U.S. Dept. of Agriculture.

Pomeranz, Y., Miller, B.S.,and Johnson, J.A. 1962. Use of lactose in breadmaking. Cereal Chem. 39:398.

Pomeranz, Y. and Finney, K.F. 1975. Sugars in breadmaking. Bakers Digest 49(1):20.

Ponte, J.G. Jr. 1978. Bread. In "Wheat Chemistry and Technology. Ed. Pomeranz, Y. A.A.C.C. Inc., St. Paul, MN. p. 675.

Ponte, J.G. Jr., Titcombs, S.T., and Cotton, R.H. 1962. Flour as a factor in bread firming. Cereal Chem. 39:437.

Pyler, E.J. 1973. Baking Science and Technology. Vol. 1 and 2. Siebel Publishing Co., Chicago, IL. pp.568, 619, 651.

Redlinger, P.A., Setser, C.S., and Dayton, A.D. 1985. Measurement of bread firmness using the Instron Universal Testing Instrument: differences resulting from test conditions. Cereal Chem. 62:223.

Reissmann, T. 1982. On the advantages of hydrolyzing ice cream mix. Nordeuropaeisk Mejeri-Tidsskrift. 48:193.

Richardson, G.H. 1985. Ed. Standard Methods for the Examination of Dairy Products. 15th ed. American Public Health Association, Washington, DC. p.133.

Richmond, M.L., Gray, J.I., and Stine, C.M. 1981. Beta-galactosidase: review of recent research related to technological application, nutritional concerns, and immobilization. J. Dairy Sci. 64:1759.

Roberts, H.R. and McFarren, E.F. 1953. The chromatographic observation of oligosaccharides formed during the lactase hydrolysis of lactose. J. Dairy Sci. 36:620.

Robinson, P.J., Dunnill, P., and Lilly, M.D. 1971. Porous glass as a solid support for immobilisation or affinity chromatography of enzymes. Biochim. Biophs Acta. 242:659.

Rothwell, J. 1979. Some recent developments in the use of milk products in ice cream. Ice Cream & Frozen Confec. 162(11):124.

Rubenthaler, G.L., Finney, P.L., Demaray, D.E. and Finney, K.F. 1980. Gasograph: design, construction, and reproducibility of a sensitive 12-channel gas recording instrument. Cereal Chem. 57:212.

Shah, N.O. and Nickerson, T.A. 1978. Functional properties of hydrolyzed lactose: crystallization. J. Food Sci. 43:1085.

Shogren, M.D., Pomeranz, Y., and Finney, K.F. 1979. Low-lactose bread. Cereal Chem. 56:465.

Shukla, T.P. 1975. Beta-galactosidase technology a solution to the lactose problem. CRC. Crit. Rev. Food Technol. 5:325.

Tang, R.T., Robinson, R.J., and Hurley, W.C. 1972. Quantitative changes in various sugar concentration during breadmaking. Bakers Digest. 46(4):48.

Vetter, J.L. 1984. Whey and whey products use in bakery applications. Proceedings, Whey Products Conference, Agricultural Research Service. U.S. Dept. of Agriculture.

Wallenfels, K. and Weil, R. 1972. Beta-galactosidase. In "The Enzyme" vol 7. Ed. Boyer, P.D. Academic Press, New York. p. 627.

Waters Associates, Inc., 1981. Sep-Pak C_{18} Cartridges - a new dimension in sample cleanup. Waters Assoc. Bull. 841, Milford, MA.

Weetall, H.H. 1975. Immobilization by covalent attachment and by entrapment. In "Immobilized Enzymes for Industrial Reactors. Ed. Messing, R.A. Academic Press, New York. pp. 108, 201, 212.

Wierzbicki, L.E. and Kosikowski, F.V. 1973. Oligosaccharides from Aspergillus

niger beta-galactosidase action on lactose. J. Dairy Sci. 56:1400.

Woollard, D.C. 1983. The estimation of glucose, galactose and residual lactose in hydrolzed whey products using cation-exchange high performance liquid chromatography. N.Z.J. Dairy Sci. Technol. 18:209.

Woychik, J.H. and Wondolowski, M.V.1973. Lactose hydrolysis in milk and milk products by bound fungal beta-galactosidase. J. Milk Technol. 36:31.

Woychik, J.H., Wondolowski, M.V., and Dahl, K.J. 1974. Preparation and application of immobilized beta-galactosidse of Sacchromyces lactis. In "Immobilized Enzymes in Food and Microbial Process". Eds. Olson, A.C. and Cooney, C.L. Plenum Press, New York, NY. p.47.

WPI, 1984. Whey products: A survey of utilization and production trends 1983. Whey Product Institute. Bull. #25 Chicago, IL.

Young, C.K., Stull, J.W., Taylor, R.R., Angus, R.C., and Daniel, T.C. 1980. Acceptability of frozen desserts made with neutralized hydrolyzed fluid cottage cheese whey. J. Food Sci. 45:805.

Zadow, J.G. 1984. Lactose: properties and uses. J. Dairy Sci. 67:2654.

Zall, R.R. 1984. Trends in whey fractionation and utilization, a global perspective. J. Dairy Sci. 67:2621.

Zarb, J.M. and Hourigan, J.A. 1979. An enzymic cryoscopic method for the estimation of lactose in milk products. N.Z.J. Dairy Sci. Technol. 14:171.

APPENDIX

APPENDIX - A

In-house sensory evaluation scoring sheet for the hydrolyzed lactose syrup breads.

Instructions: Please feel, smell, and taste the samples of bread in the given order and indicate how much you like or dislike each one. You may use any of the descriptive terms below to complete the general comment section.

BREAD SAMPLES

PARAMETERS	1	2	3	4	5	6	7
Like very much (7)							
Like moderately (6)							
Like slightly (5)							
Neither like nor dislike (4)							
Dislike slightly (3)							
Dislike moderately (2)							
Dislike very much (1)							
GENERAL COMMENT							

Texture - smooth, soft, velvety, coarse, tough, etc.

Flavor - sweet, pleasant, sharp, whey or taint-like, sour, etc.

Grains - large, small, open, irregular, uniform, etc.

PREPARATION OF HYDROLYZED LACTOSE SYRUP FROM WHEY PERMEATE AND ITS FUNCTIONAL PROPERTIES IN WHITE PAN BREAD FORMULATION

by

OLUYEMI A. OGUNRINOLA

B.S. University of Illinois, 1983

AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

β-Galactosidase from Aspergillus oryzae was covalently attached to glass beads for an immobilized enzyme preparation. The immobilized enzyme was then utilized to hydrolyze lactose in sweet permeate (from Cheddar cheese whey processed with an ABCOR-Sanitary UF pilot system) in a continous flow reactor at 50°C. The hydrolyzed whey permeate (HWP) was prepared into three batches based on the degree of lactose hydrolysis (50, 75, and 95%). Each of the variables was also classified as "demineralized" and "undemineralized", and the latter was treated accordingly with ion exchange system. All batches were decolorized and concentrated into syrups at 50-65°C under vaccum. Syrups were used at 6% flour basis (700g flour) to substitute sucrose in a typical white pan bread formulation.

The experimental and control doughs and/or breads were evaluated on some specific physical and chemical characteristics that include: fermentation rate, pH, specific volume, texture, grain, break and shread, firmness, residual sugars, and in-house sensory analysis.

The lowest and highest level of lactose hydrolysis (50 and 95%) had textural effect on the doughs. The 95% hydrolysis appeared to have no significant functional benefit for the breads. The undemineralized HLS, in general, produced slightly drier doughs. The demineralized 75% hydrolyzed syrups breads were considered the best in overall evaluation, followed by the control. The demineralized 75% hydrolyzed syrups breads showed the least firmness during the testing periods. There was no outright rejection of the hydrolyzed lactose syrups breads by the panelists. A higher proportion of the panelists preferred the demineralized 75% HLS breads than any other variables. The residual sugar content of the experimental doughs were similar

in amount and number of sugars detected with the exception of the 75% HLS breads which showed two unknown peaks and a higher proportion of maltose.