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Use of Dry Whey and Lactose Hydrolysis in Yogurt Bases

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USE OF DRY WHEY AND LACTOSE HYDROLYSIS IN YOGURT BASES

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

Yogurt is one of the oldest and most traditional fermented dairy products. Since early times it has been an important food item of the people in the Middle East. Except for its refreshing taste and wholesomeness as a food, no special virtues were claimed for it until early in the 20th century when the bacteriologist, Elie Metchnikoff, who shared a Nobel Prize in 1908, concluded from his studies on the effect of lactic acid bacteria of the digestive tract, that yogurt arrests putrefaction in the intestinal tract and thus might be beneficial to health (55).

Attempts to popularize yogurt in the United States (US) and Canada were first successful in the 1940's. In 1955, the total production of yogurt in the US was only 17,000,000 lb, whereas by 1980 the production increased to 589,000,000 lb. On a per capita basis, consumption rose from .2 lb in 1960 to 2.67 lb in 1980 (64). The future looks bright for the yogurt industry, particularly in view of the fact that per-capita consumption in the US is still far below that of most European countries; annual yogurt consumption per person in 1977 was 1.2 kg in the US compared to 14.9 kg in the Netherlands, 14.2 kg in Denmark, 12.2 kg in Switzerland, and 8.0 kg in France (103).

Whey is plentiful. According to Delaney (19), approximately 16,000,000 tons of whey are produced in the US yearly. About 80% of the whey is from whole milk cheese and 20% from cottage cheese manufacture. It is estimated that just over one-half of this whey is

used and the remainder is disposed as waste (6). In the middle ages, whey was utilized as a pharmaceutical drug, as a skin balm, and in cattle feed; but rarely was it used as a food for humans. As the cheese industry grew, production of an increasing volume of byproduct fluid whey, for which there was little demand, accompanied it. Strong new regulations prohibit dumping of whey into streams, rivers, and even into municipal sewerage systems because of its high biological oxidation demand (53).

The dairy industry is always interested in use of new and different ingredients that are lower in cost and do not affect quality of product. A great deal of research has been aimed toward promoting proper utilization of whey; but it has not been nearly enough, and utilization of whey remains perhaps the most serious problem facing the dairy industry worldwide. One must therefore admire the many efforts in research and manufacturing aimed at making something consumable and marketable, if not profitable, from whey. The use of whey in yogurt and other dairy products has been limited heretofore, because of its effect on the quality of the finished product. However, in the current decade research has been done on the feasibility of replacing nonfat dry milk with dry whey in yogurt and frozen desserts.

Use of lactase (β-D-galactosidase or E. C. 3. 2. 1. 23 β-D-galactoside galactohydrolase) to hydrolyze lactose, the major carbohydrate of milk, into its constituent monosaccharides glucose and galactose prior to product manufacture has received considerable

attention during the past decade. Applications for the food industry are readily apparent; one application is preparation of low-lactose dairy products intended for use by lactose sensitive individuals.

The objectives of this research were: 1) to determine the acceptability of yogurts made with reconstituted nonfat dry milk bases, having 50 or 75% hydrolysis of the total lactose available in the mixes along with replacement of 25 or 50% of the nonfat dry milk content with sweet dry whey; 2) to ascertain economy achieved by use of dry whey, which costs less than nonfat dry milk, and use of less sugar in hydrolyzed batches since the products of lactose hydrolysis are sweeter than lactose per se; and 3) to ascertain whether enzymatic hydrolysis of lactose to its component simple sugars would make possible the use of greater percentages of dry whey in yogurt formulas without adverse effects on flavor and/or other properties of the yogurt.

LITERATURE REVIEW

Yogurt is a coagulated milk product obtained by lactic acid fermentation through the action of Lactobacillus bulgaricus and Streptococcus thermophilus, with one or more of other optional ingredients such as nonfat dry milk, whey, buttermilk, carbohydrate sweetener, flavoring ingredients, color additives, and stabilizer. The food may be homogenized and shall be pasteurized or ultra-pasteurized prior to the addition of bacterial culture and bulky

flavorings materials. To extend the shelf-life of the food, yogurt may be heat-treated after culturing is completed to destroy viable microorganisms (24).

Lactose Sensitivity in People

Studies in recent years have demonstrated a pattern of milk intolerance in non Caucasian children and adults which has been attributed to low levels of intestinal lactase. This condition may occur in infants on a congenital basis and may appear in adults secondary to intestinal damage or as a late manifestation of an inherited condition (75). In the US, 70% of the adult black population and about 10 to 15% of adult Caucasians are afflicted with this condition (87). It has been estimated that approximately 70% of the world's adult population is lactose intolerant (39, 121). In some developing countries, the incidence of lactose intolerance can be much higher. In fact, the incidence of lactase deficiency is about 95% for Asians (87). This may be due to an adaptive decline in the enzyme following withdrawal of the milk from the diet as the child grows older (84, 85). In addition to the gastrointestinal discomfort brought about by ingestion of milk by these individuals, a general impairment in the normal digestion process has been observed (39).

Yogurt Manufacture

Traditionally, yogurt has been manufactured from milk

concentrated by boiling. Although whole milk is often the only dairy ingredient required, skim milk may be blended to give a low fat and a high total solids content. These blends may be increased in total solids to 15 to 17% by fortifying with 2 or 3% nonfat dry milk (51). The levels of total solids in the milk are significant for both the consistency and aroma of the manufactured yogurt. An increase in the total solids will enhance these properties. Most yogurt currently marketed in the US contains from 12 to 15% milk solids (12). The total solids levels in the milk for yogurt manufacture can vary from as low as 9% in skim milk yogurt to over 20% in other types of yogurt. The recommended range is from 14 to 18% and the best yogurt is made from milk containing 15.5 to 16.0% total solids (103). The level of total solids also affects the titrable acidity of the mix due to the buffering action of the proteins, phosphates, citrates, lactates, and other miscellaneous milk constituents (45). An increase in total solids results in an increase in the titrable acidity and reduction in the coagulation time (14).

For production of good quality yogurt, an excellent raw milk supply is of primary importance. Conditions under which the raw milk is collected, stored, and handled can greatly influence its quality in terms of flavor. Any off-flavors in the raw milk can be carried over into the finished products (13).

Nonfat dry milk is widely used in the industry to fortify fluid milk for production of thick smooth yogurt. The level of

fortification varies from as little as 1% to as much as 6%. However, the generally recommended level of fortification is around 3 to 4% (9). Ultrafiltration (UF) and reverse osmosis (RO) also may be used to achieve higher solids contents. Concentration of milk by UF and RO is carried out at ambient temperatures or slightly above, and hence, avoids chemical damage of milk constituents caused by heating. The application of these methods in cultured dairy products has been reported by Jespen (46) and Kosikowski (52). Milk concentrated by UF to 18 to 20% total solids has been reported to produce good quality yogurt without the need for homogenization (103). Granier (30) and Emaldi et al. (20) found yogurt made from UF concentrated skim milk with the lactose adjusted to 2% was superior to ordinary commercial yogurt.

The viscosity of yogurt is almost wholly dependent on the protein content of the milk. Hence, a high protein concentration is essential for production of a viscous yogurt. Casein is the major contributor of viscosity followed by fat and albumin (8).

Stabilizers, like milk solids, can also influence the consistency of yogurt. In practice, gelatin, starch, vegetable gums, and pectin receive the widest use as stabilizers for yogurt (13, 100). The quantity of stabilizer used is indicated by the stabilizer system selected and the end product consistency sought (55). Kroger (57) stated the best yogurt texture is achieved by using gelatin at .3 to .8%. Agar and pectin were found to produce satisfactory thickening but delayed acid production (14). In Europe,

gelatin, agar-agar, and pectin in amounts of 1.0 to 3.0%, are generally used (14). Good yogurt can be made without the use of added stabilizers (51), but a yogurt without stabilizer is more vulnerable to a number of stress factors than one that has been stabilized properly. When properly chosen and used, stabilizers play an important role in improving the body, texture, mouth-feel, and appearance of yogurt (57).

The presence of milk fat in the yogurt mix affects the mouth-feel of the product. The higher the milk fat content, the smoother the product texture will be. The fat content may vary from .5 to 3.5% in yogurt (67). In a study by Kroger and Weaver (59) in Central Pennsylvania area, the fat content of 44 yogurt samples varies from .82 to 2.04%. Optimum milk fat levels are between 2 to 4% (13). A milk fat level of 3.0 to 3.5% for plain yogurt was recommended by Manus (61). According to Morley (67), 2% fat milk gives the best yogurt drink for flavor and body and still allows the product to be called low fat yogurt drink. Milk fat also tends to "mask" the acid flavor of the yogurt. Obviously, when milk fat is incorporated into a mix formulation, homogenization becomes important to the overall texture quality of the yogurt.

Addition of sugar is a method of cutting the sharpness of yogurt flavor. Enough sugar should be used to mask the full degree of acidity, but enough tartness should remain for a desirable acid-sugar blend. If the final pH is controlled to 4.2 to 4.0, 4 to 6% sugar will be sufficient to give a desirable blend (61). Sucrose

up to 10% is usually used when fruit is added to yogurt (14). Bills et al. (10) found a mix containing 4% or more sucrose decreased acid production and lowered cell counts of both microbial species. Acetaldehyde production was lowered in mixed cultures grown in media containing 8% or more sucrose.

As yogurt mix is prepared, particular attention should be given to blending, homogenization, and heat treatment of the mix. The blending and homogenization steps are important to the uniformity of ingredient distribution. These steps do not present any serious process problems. Homogenization is usually carried out before the heat treatment, but in some cases it may take place after the heat treatment. The homogenization process splits the fat globules into smaller globules which become coated with a new membrane comprised largely of casein submicelles (14). The process effectively increases the density of the fat globules and reduces their tendency to agglutinate (71); and the fat becomes evenly dispersed through the liquid and does not separate out during incubation in yogurt manufacture (103). Homogenization also tends to reduce syneresis (55, 57). Yogurt viscosity is dependent on both the temperature and the pressure of homogenization, with the best results being achieved at 2000 to 2500 psig and at 50 to 60^{o}C (71).

The heat treatment of the mix is the first critical step in the process. The primary aim of the heat treatment in yogurt manufacture is destruction of microorganisms which may be pathogenic or which may adversely affect the quality of the product. Almost all

organisms, with the exception of some sporeformers in the vegetative forms, are destroyed during yogurt manufacture (103). When good quality dairy products are used, the number of bacteria surviving this heat treatment is small and they are restricted in growth during incubation by the rapid acid formation (35).

Heat treatment of yogurt mix also plays a critical role in ultimate body and texture. The recommended heat treatment for a yogurt mix is 82 to 90°C for 30 min (13, 35, 51, 61, 67, 104). If the 90°C temperature is exceeded for 30 min, changes develop that favor syneresis in the finished product (13). Low heat treatment is a factor in thin body and wheying-off (55). The heat treatment given to the mix dictates the denaturation rate and the degree of denaturation of whey proteins. This heat treatment denatures the whey proteins and even alters the casein to a limited extent. In particular, β-lactoglobulin is almost completely denatured. The interaction between the denatured β-lactoglobulin and the casein increases the hydrophilic properties of the casein and facilitates the formation of a stable coagulum (14, 61). It has been found that the hydration effect of the protein is maximal when milk is heated at 85°C but decreases as the temperature is raised above 85°C (103). Too much heat treatment of the mix results in a loss of water binding capacity of whey proteins; as a result, syneresis develops and the gel structure of the yogurt curd becomes weak and fragile (13).

The amounts of denatured whey protein and casein-whey

protein complexes are largely dependent on the composition of the yogurt mix. The preceding remarks apply only to milk per se; nonfat dry milk, which commonly is used for fortification, has already undergone heat induced changes during the different stages of forewarming, concentration, and drying and may react quite differently during the heat treatment (103). Optimum viscosity can be reached in high solids mixes without complete denaturation of whey proteins. The whey proteins are protected from denaturation in the presence of high solids content (74).

After the mix has been heat-treated, the mix is cooled to optimum inoculation temperature, usually about 45°C (35, 51, 104), followed by introduction of 2 to 5% appropriate liquid culture. The inoculated warm mix is dispensed into consumer containers or stainless steel vats and incubated at about 41 to 43°C (13, 35) for 3 to 6 h, depending on type of culture (67).

A new trend in yogurt making, whereby yogurt mixes are incubated at significantly lower temperatures, usually 30 to 32°C for 12 to 14 h, has been reported by Kosikowski (51) and Chambers (13). Incubation at lower temperatures tends to favor slower acid development and improved curd formation.

The starter culture for yogurt consists of Lactobacillus bulgaricus and Streptococcus thermophilus. It has been observed that a high quality yogurt with a pleasant taste depends very much on the ratio of the two bacterial species present. The Streptococcus:Lactobacillus ratio in the final product should be 1:1 and

not above 3:2 for optimum results (61, 97). It can be adjusted by controlling incubation time and temperature. The ratio should be checked by microscopic examination. The S. thermophilus grows better at 37°C, whereas, L. bulgaricus grows more rapidly in the 44 to 46°C range (110). Incubation temperatures of 41 to 42°C tend to favor the Lactobacillus and Streptococcus cultures equally and yield the desirable 1:1 ratio with some degree of reliability (13). If the incubation temperature is increased above the 42 to 46°C range, the Lactobacillus culture is favored and will predominate. In contrast, if the temperature is decreased below 41°C, the Streptococcus culture is favored and ultimately culture domination can occur (110). It is the incubation part of making yogurt where science becomes art, where the trial-and-error approach is often as fruitful as the strict control of all variables (55).

The flavor of yogurt depends largely upon the culture organisms and their metabolism during incubation. Off-tastes and off-odors are usually byproducts of faulty fermentation. The characteristic flavor of yogurt is due to lactic acid, which has no odor of its own, and trace amounts of acetaldelyde, diacetyl, and acetic acid (18). Acetaldehyde is produced primarily by the 'coccus', S. thermophilus, in the early stages of incubation. The typical high acid flavor is produced mainly by the 'rod', L. bulgaricus, later in the incubation period. Neither organism alone is capable of producing both flavor components in the desired amounts (65). Yogurt is thus a product of bacterial symbiosis.

After inoculation, the streptococci grow fastest until about pH 5.5, then the growth of lactobacilli is progressively favored. Lactobacilli culture produces proteases which liberate essential amino acids, especially valine, required by streptococci (13, 18, 97, 103). If the incubation is not halted at a pH between 4.0 to 4.4, the lactobacilli would continue to grow. Since they are capable of producing acid as well as flavor, the acidity would go well below pH 4.0; the streptococci would disappear, the optimum bacterial ratio would be upset, and the product would be extremely sour (18, 55, 57, 110).

Production of lactic acid is the most important chemical process which occurs during yogurt manufacture. The lactic acid helps to destabilize the casein micelle and this leads to coagulation of milk protein and formation of the yogurt gel (103).

The major flavor compounds produced by lactic culture organisms include acetaldehyde, diacetyl, acetone, ethanol, and acetoin. The presence of acetaldehyde is important for good yogurt flavor (47, 88).

When the desired titrable acidity is reached, the yogurt must be cooled rapidly to below 26^{o}C and then properly cooled to 4^{o}C with little or no agitation. Rapidly cooling to below 26^{o}C arrests the acid development and begins conditioning the protein for better whey retention (13). A final acidity of .95 to 1% is desirable (67). DeHaast (18) recommended a final titrable acidity of 1.0 to 1.25%.

Whey Composition and Properties

Whey is the watery portion or serum that separates from the curd during conventional cheese making or casein manufacture. Whey may be considered as milk which has had the major milk protein (casein) and milk fat removed (117). It constitutes about 85 to 90% of the volume of the milk used for transformation into ripened cheese, and it retains about 55% of the milk nutrients (53). These, among the best that milk can offer, include minerals, vitamins, lactose, and the proteins lactalbumin and lactoglubulin (15).

From cheddar type cheese the whey is sweet, pH 5.9 to 6.3; but from unripened cottage type cheeses they whey is acid, usually pH 4.4 to 4.6. There is more lactic acid, calcium, phosphorus, and less lactose in acid whey and less acceptance by consumers because of the acid flavor (51). This makes it difficult for acid whey, in any form, to enter the standard channels of whey utilization.

Milk sugar (lactose), the most abundant ingredient of whey solids, is a unique sugar which plays a major role in the assimilation of calcium and phosphorus (15, 63). Lactose acts as a carrier for flavor and color when added to many foods. It has physical and chemical properties that give it distinct advantages over other sugars in certain foods and pharmaceutical applications. Nutritionally, lactose is a source of galactose, the structural sugar needed for repair of brain, mucous, and other delicate tissues (63) and of glucose, the sugar of blood necessary for brain function and tissue metabolism.

Whey is an excellent source of milk minerals such as calcium, potassium, sodium, and certain trace elements. It is also a rich source of water soluble vitamins of the B-complex (riboflavin, pantothenic acid, thiamine, and niacin) (15, 114).

Whey protein is one of the highest quality naturally occurring proteins, having a protein efficiency ratio (PER) of 3.0 to 3.2 compared to casein at 2.5 (63, 89). Whey proteins have adequate levels of essential amino acids and are easily digestible, and so are highly nutritional and physiologically complete (15, 92). However, vegetable proteins lack one or more essential amino acids such as lysine or tryptophan. Such deficiencies are deterrents to protein utilization in vivo. Nutritional and biochemical studies of whey products have been reported extensively by Forsum and Hambraeus (25) and Glass and Hedrick (27, 28).

Whey proteins are relatively susceptible to denaturation (68). More than 90% of the whey proteins are coagulated when milk is heated above 93^{o}C for a few minutes (114). The old method of separating whey protein from whey by heat treatment is simple, but it yields a product which may be gritty, insoluble, and lacking in functionality, since the protein is denatured in this process (3). Production of undenatured whey protein concentrates as well as other modified whey products has been reported by Kosikowski (53), Delaney (19), Weisberg and Goldsmith (117), and Webb (113).

Modified wheys such as partially delactosed whey, partially demineralized whey, and whey protein concentrate have been used

successfully to contribute to the milk solids-not-fat content of ice cream and other frozen desserts. The addition of modified whey solids to ice cream is said to improve texture and freezability while giving an acceptable flavor and sweetness (3).

Whey solids also have been found to be highly beneficial in the preparation of infant formulations. The addition of dry whey to these formulations produces a "humanized" milk that is much closer in composition to mother's milk than the bovine milk (62, 92). Whey solids are also used in other dairy products, especially in ice cream and sherbets. United States Federal regulations permit addition of dry whey up to 25% replacement for milk solids-not-fat. This amount apparently retains the basic quality of the product (62, 63, 116). Whey has been found to be useful as a base for producing soups and gravies, as well as starter culture to make yogurt, sour cream, buttermilk, and cheese (3, 92). Whey also is used in combination with milk to make ricotta and certain processed cheeses (50). The manufacture and attributes of whey beverages have been extensively reviewed by Holsinger et al. (40). Whey powder adds a natural tenderness to many products. The lactose and lactoglobulin help to produce tenderness in biscuits, pie dough, crackers, and other baked products without the addition of extra shortening (15, 117).

The total solids content of nonfat dry milk (NDM) and dry whey is the same. The lactose and mineral contents in dry whey are nearly one and one-half times greater than those of NDM

(Table 1). However, the protein content of dry whey is substantially lower (6, 72). Hence, the nutritional contribution of dry whey is less than that of NDM, as is its contribution to body and texture. So, NDM can be replaced with dry whey only to a certain extent; the actual amount depends upon the usage.

TABLE 1. Comparison of composition of dry whey and nonfat dry milk.[a]

Constituents	Approximate content	
	NDM	Dry whey
	%	
Casein protein	30.0	Nil
Lactalbumin	6.0	13.0
Lactose	51.0	71.0
Fat	.5	.87
Ash	8.2	11.0
Moisture	4.0	4.0

[a]Source; (15).

Uses of Whey in Yogurt

Several workers have investigated the possible reduction of NDM in yogurt by using whey solids instead. Hartman (36) prepared yogurt formulations containing neutralized (pH 6.55) liquid cottage cheese whey or sweet, acid, and various modified dry wheys and found that as much as 2% whey solids from these sources could be incorporated without producing a distinct whey flavor. To maintain

good body and to support bacterial growth for adequate acid development, he found it needful to add enough NDM to bring the total nonfat milk solids to a minimum of 9.5%. Yogurt culture did not grow as well in yogurt with added dry acid whey or concentrated whey. Apparently, the acid or other inhibitors prevented normal growth. "Whey" off-flavor, not typical of yogurt made from fresh milk ingredients, and weak body are the quality factors which limit the use of whey in yogurt. It was concluded that sweet whey solids or neutralized cottage cheese whey solids can be used in yogurt at the rate of 1 to 2% to replace an equivalent amount of nonfat dry milk without affecting body, providing total milk solids-not-fat are not below 9.5%.

Todoric and Savadinovic (108) added .2 to .6% dry whey to a yogurt base containing 3.2% fat which already had been pasteurized at 90°C and stored at 4°C for 18 h. After addition of dry whey, the mix was pasteurized at 82°C for 15 min and homogenized at 2,500 psi, inoculated with 2% culture and incubated at 42°C for 3 h, followed by cooling and storage for 5 days at 4 to 6°C. Addition of dry whey increased viscosity of the yogurt and enhanced acid development during incubation and storage. Results of organoleptic tests showed that samples with .4% dry whey lacked specific yogurt aroma, gave a sweet off-flavor, and exhibited whey separation on storage. The investigators concluded that a maximum of .3% dry whey could be used in place of nonfat dry milk.

Prodanski (83) made yogurt from milk fortified with

proteins which had been recovered from whey and buttermilk. The recovery technique involved acidification to pH 4.8 to 4.9, addition of .025 to .030% calcium chloride, heating to 80 to 85°C and holding until the coagulation of proteins was complete, then washing at 12 to 14°C for 18 to 20 h. The resultant product contained 28 to 35% total solids and was incorporated into raw milk at the rate of 2 to 6%. The products made with the addition of whey and/or buttermilk proteins were said to have good consistency and flavor.

Application of liquid whey in the manufacture of yogurt was reported by Jelen and Horbal (44). They prepared a base by reconstituting nonfat dry milk in liquid cottage cheese whey which had been adjusted to pH 6.2 to 6.4 by sodium hydroxide or sodium bicarbonate and with or without fresh homogenized milk. Mixes containing 15 and 20% total solids could be pasteurized at 82°C for 3 min with only slight increase in viscosity. The yogurt was incubated at 45°C for 4 to 6 h. A satisfactory product was made from 60% cottage cheese whey, 29% homogenized milk, and 11% nonfat dry milk. Firmness of yogurt increased with increased total solids and increased proportion of fresh homogenized milk.

Korner (49) prepared yogurt from pasteurized whole milk or skim milk or whey which had been circulated at approximately 4 atm absolute pressure through a semi-permeable membrane to separate the protein from other milk constituents. The resulting protein was claimed to be free of microorganisms and possess natural flavor. Approximately four parts of untreated whole milk or skim milk were

mixed with one part of milk enriched with milk protein so it contained 10% protein but no salts or sugars. Firmness of yogurt was increased by increasing the content of protein concentrate.

Utilization of acid whey in frozen yogurt was reported by Hekmati and Bradley (37). They prepared soft-serve frozen yogurt containing up to 43.39% fluid acid whey and they claimed that use of acid whey resulted in a finished product containing higher total solids, improved body and texture, and higher nutritional value.

The characteristics of dry, acid whey from cottage, bakers', and cream cheese offer special opportunities for application in cultured products with high acidity such as sour cream, cheese, and other flavored dips, dairy spreads, and fruit yogurts. The natural, cultured, fermented flavor of acid whey is unique and can not easily be simulated by artificial acidulants. This flavor blends easily with and accentuates most fruit flavors (73).

Lactose and its Enzymatic Hydrolysis

Lactose is the major sugar that comes from mammalian sources. Lactose in foods has a variety of sources such as milk, cream, nonfat dry milk, whey solids, modified whey products, or refined lactose (69). More than 70% of whey solids are lactose, which is one and one-half times the amount of lactose in NDM. Lactose has the capacity to accentuate flavors and contributes sweetness. Recent high prices of sucrose have aroused new interest in edible lactose as a carbohydrate filler to improve the body and

mouth-feel of many foods, and also as a humectant (41).

Lactose is a potential source of sweetness, but it is no match for sucrose and other sugars in this respect (Table 2). It requires 2.5 to 3.5 times as much lactose to get the same sweetness

TABLE 2. Relative sweetness of sugars.[a]

Percent concentration to give equivalent sweetness			
Sucrose	Glucose	Fructose	Lactose
1.0	1.8	.8	3.5
2.0	3.6	1.7	6.5
5.0	8.3	4.2	15.7
10.0	13.9	8.6	25.9

[a]Source: (71)

effect as sucrose (71). The relative sweetness of lactose is 16 as compared to a sucrose value of 100 (5) (Table 3). The relative sweetness of sugars depends on many factors such as pH, temperature, and other constituents. The relative sweetness also changes with concentration (90).

Lactose is far less soluble in water than is sucrose. Maximum lactose solubility at room temperature under equilibrium conditions is only 18% compared to about 68% solubility of sucrose (41). Hence, lactose utilization often is limited by its low solubility, lack of sweetness, and its laxative effect if consumed in large amounts (118). Apart from this, the high lactose content in non

fermented milk products such as ice cream and condensed milk products poses technical problems attendant to preventing excessive lactose crystallization (93).

TABLE 3. Approximate relative sweetening values of sugars as compared with sucrose value of 100.[a]

Sugars	Sweetening value
Fructose	173
Invert sugar (glucose & fructose)	127
Sucrose	100
Glucose (dextrose)	74
Galactose	32
Maltose	32
Lactose	16

[a]Source: (5)

These limitations of lactose are greatly minimized by hydrolyzing the sugar with the enzyme lactase. The resulting sugars, glucose and galactose, are known to be sweeter than lactose itself (5, 90) (Table 3). Hydrolysis of the lactose in milk and whey results in several changes in their physical and chemical properties of value to the dairy manufacturer. These changes include reduced lactose content, little or no lactose crystallization, increased carbohydrate solubility, increased sweetness, and more ready fermentation of the sugars (39).

Applications of enzymatic hydrolysis of lactose are numerous, not only for producing milk and whey products with modified physical and functional properties but also for providing low lactose dairy products for lactase deficient individuals. Low lactose or lactose-free milk for lactose sensitive individuals can be prepared either by physical removel of the lactose by ultrafiltration or by hydrolysis of lactose into the corresponding monosaccharides glucose and galactose. The latter method is preferred, both because of the taste and because of the considerable losses of energy, minerals, and vitamins which accompany lactose removal (16). Upon hydrolysis, the sugar mixture is more soluble, sweeter, more easily digestible by the lactose intolerant, and fermentable by a greater variety of microorganisms (76).

Lactose can be hydrolyzed using strong mineral acid, ion exchange resins, or enzymes (69, 76). Acid and ion exchange resins tend to ruin the functionality of the whey proteins through irreversible denaturation. The use of hydrolyzing enzymes has the advantage of lowering the lactose content without adversely affecting the proteins and other components (76).

The enzyme β-galactosidase or β-D-galactoside galactohydrolase (E. C. 3.2.1.23), commonly called lactase, catalyzes the hydrolysis of β-galactosidic linkages such as are present in lactose (11, 76, 81, 93, 111). Beta-galactosidase can be isolated from plant, animal, and microbial sources. It occurs naturally in kefir grains,

almonds, tips of wild roses, seeds of alfalfa, soybeans, and coffee (81, 93, 122). The enzyme has been found in the fungi, Aspergillus niger, Aspergillus oryzae, and Aspergillus flavus. The enzyme has also been obtained from various strains of Lactobacilli, Streptococcus thermophilus, and Escherichia coli. The lactase activity of Lactobacillus bulgaricus and Streptococcus thermophilus has been reported by Kilara and Shahani (48). Yogurt prepared by direct acidification process did not possess lactase activity. Cultured yogurt possessed considerable enzymic activity mainly due to lactase as an endoenzyme in the yogurt culture. S. thermophilus contained approximately three times more lactase than did L. bulgaricus. Enzyme concentration increased with time of incubation.

Although lactase enzyme is found in various sources, the microbes (bacteria, yeast, and fungi) offer higher yield for commercial production (93). The isolation and purification of the enzyme have been reviewed by Shukla (93), Pomeranz (81), and Borglum and Sternberg (11). The enzyme has been prepared commercially from Aspergillus niger, Saccharomyces lactis, and E. coli (93). The enzymes from Aspergillus and from Saccharomyces appear to be the most useful for industrial exploitation because of ease of extracting the enzymes, properties of the enzymes, and acceptance of Aspergillus and Saccharomyces enzymes in processing of foods (11).

These lactases differ widely in their properties, particularly in pH optima. Lactase from S. lactis has optimum activity at pH 6.8 to 7.0, is stable in the pH range 6.0 to 8.5, and works best

at a temperature of 35°C. Although it is suitable for treating milk (pH 6.6) and sweet whey (pH 6.2), the lack of stability below pH 6.0 precludes its use in treating acid whey (pH 4.5). A. niger lactase, with a pH optimum of 4.0 to 4.5, good stability over a wide pH range (ph 3.0 to 7.0), and an optimum temperature of 55°C is suitable for the lactose modification of acid whey (39, 124). The two commercial lactases used today are "Lactase LP" from A. niger and "Maxilact" from S. lactis.

Guy and Bingham (31), and Dahlqvist et al. (16) used lactase from Saccharomyces lactis in skim milk and wheys to determine optimum conditions for converting lactose to monosaccharides. The optimum pH for lactose hydrolysis was 6.5 to 6.8, which coincides with the pH of normal milk. The lactase had only moderate temperature stability, for it was rapidly inactivated above 35°C. Heating lactase for 1 min resulted in 97% inactivation at 60°C and complete inactivation at 70°C. Lactose could be hydrolyzed in 22 h at 5°C as effectively as in 2 h at 31°C (31). Potassium, magnesium, and manganese ions slightly accelerated lactase activity in fluid milks while sodium and calcium ions inhibited the reaction significantly.

Wendorff et al. (119) found milk solids, other than lactose, either inhibit or suppress β-galactosidase activity in milk products. Of all the milk products tested, whey was the best substrate for the enzyme. The maximum rate of lactose hydrolysis in milk products was obtained when milk or whey was fortified with

potassium and magnesium ions. Hydrolyses proceeded rapidly to 50%, more slowly to 70%, and was negligible beyond 75% conversion (82).

Wierzbicki and Kosikowski (122) evaluated 23 strains of molds, yeasts, and bacteria for lactase activity and cell yield. They concluded the bacteria produced highest lactase activity and lowest cell yields; whereas the molds had the lowest lactase activity but the highest cell yields.

Theoretically, when lactase enzyme hydrolyses lactose, glucose and galactose are produced in equal amounts. However, in practice this does not happen, because other sugars (oligosaccharides) are formed in addition to glucose and galactose. Formation of oligosaccharides has been reported by Wierzbicki and Kosikowski (123), Shukla (93), Toba and Adachi (107), Roberts and Pettinati (86), and Pazur (79). Beta-galactosidase splits glycosidic linkages of lactose to produce glucose and galactose and may transfer some monosaccharide units to active acceptors, such as monosaccharides, polysaccharides, or alcohol (93, 123). The process is called trans-galactosidation and is subject to both enzymatic and chemical catalysis which leads to the formation of oligosaccharides of varying length and molecular weight. The number or types of oligosaccharides formed are affected by the substrate concentration, the source of enzyme, the pH, the temperature, and the nature of the substrate (86, 93, 123). Toba and Adachi (107) found that with S. fragilis β-galactosidase, twelve oligosaccharides were formed; while with A. niger β-galactosidase, ten oligosaccharides were

formed. The significance of oligosaccharides in high concentrations in food may be important nutritionally because of man's inability to digest them (123). However, Vujicic et al. (112) found there was no oligosaccharide formation during acid hydrolysis; if formed at all, they were in small amounts as compared to enzymatic hydrolysis.

Lactose-Hydrolyzed Yogurt

Although commercial production of yogurt from lactose hydrolyzed milk is not widely done, research work in this field has been reported by many workers. Gyuricsek and Thompson (34) prepared yogurt from 0, 25, 50, 75, and >90% hydrolyzed lactose milks fortified with 4% NDM. The time required to reach the desired pH value of 4.6 was reduced by 40 min and acid flavor of yogurt was found to be partially off-set by the sweetness imparted by glucose. Hydrolyzed lactose yogurts were preferred over plain yogurt in a comparative evaluation and were also smoother in body. It was concluded that the consumption of hydrolyzed lactose yogurt would reduce the lactose intolerance reaction, improve the overall nutrition of the consumer, and could result in increased sales of the products.

In another study, Thompson and Gyuricsek (106) noted a reduction in time required to reach pH 4.6 in yogurts from lactose hydrolyzed milks. Yogurts prepared from 90 to 95% hydrolyzed lactose were sweeter than the control and had a more acceptable flavor to persons who normally did not eat yogurt.

O'Leary and Woychik (78) made yogurts from 70 to 75%

lactose hydrolyzed skim milk fortified with 4% nonfat dry milk. Faster acid development in lactose-hydrolyzed yogurts was reported; too, less time was required for the pH to decrease to pH 4.6 in lactose hydrolyzed milk than in the control. It was concluded the faster acid development in yogurts prepared from lactose-hydrolyzed milk was primarily due to an acceleration in the initial rate of acid production when the lactose was prehydrolyzed. The lactose-hydrolyzed yogurt contained more lactic acid than did the control yogurt at pH 4.6. The greater quantity of lactic acid produced by the yogurt starter organisms in lactose-hydrolyzed milk may have been due to an alteration in the pattern of metabolites produced resulting from the utilization of a greater proportion of total available sugar in the form of glucose. It was observed that when galactose served as the energy source, a much smaller proportion of the sugar was converted to lactic acid and a proportionately greater amount was converted to acetic or formic acid and ethyl alcohol than when glucose served as the energy source. It was found that twice as much galactose was metabolized in control milk as in lactose hydrolyzed milk. In flavor evaluation of the two yogurts by a sensory panel, the lactose-hydrolyzed yogurt was scored significantly higher than was the control due to substantially sweeter character of the former resulting from the presence of free glucose and galactose. Goodenough and Kleyn (29) reported that the yogurt microflora will hydrolyze additional lactose if in the milk to provide additional glucose for metabolism but galactose continues

to accumulate in the product at a rate consistent with lactose hydrolysis.

O'Leary and Woychik (77), in another experiment, found acid development was more rapid in lactose-hydrolyzed milk. They observed glucose was utilized throughout the incubation period, whereas, lactose utilization took place only up to 4 h with the most rapid period of utilization occuring between 2 and 4 h. Free galactose was not utilized.

Tamime (102) prepared yogurt from lactose hydrolyzed milk and reported starter culture was most active in 50% hydrolyzed lactose milk as compared to growth in unhydrolyzed or 100% hydrolyzed lactose milk. Patterns of acid development of starter culture increased in 100% hydrolyzed lactose milk as compared with the unhydrolyzed and reached the optimum in 50% hydrolyzed lactose milk. The reduced activity in 100% hydrolyzed lactose milk could be attributed to production of other metabolites besides lactic acid. It was mentioned that incubation could be reduced by as much 1 h without adverse effect on the consistency of the yogurt. The increased activity of the starter culture was attributed to the availability of free glucose.

Reporting from another experiment, Tamime (101) indicated acid production by starter organisms increased in 100% hydrolyzed lactose milk as compared with acid production in milk without lactose hydrolysis; and optimum activity was reported in 50% hydrolyzed milk. It was concluded that if the lactose hydrolysis is as high

as 100%, the product becomes slightly insipid to yogurt "lovers". Yogurt produced from milk with 60% or more lactose-hydrolyzed was reported sweet by Engel (21). It was also reported that excess lactose could be hydrolyzed for the production of sweet yogurt with no increase in calorific value.

The manufacture of yogurt by simultaneous hydrolysis-fermentation was reported recently. Hilgendorff (38) used fungal lactase derived from Aspergillus oryzae to hydrolyze lactose in milk during yogurt manufacturing. It was reported the coagulation time was shorter for yogurts made with lactase. The organoleptic preference was similar to that for yogurt prepared from pretreated hydrolyzed milk. The residual lactase had added advantages for digestive qualities. In a similar type of study by Dariani et al. (17), the enzyme was added simultaneously with the starter culture for manufacturing yogurt. Out of five different brands of lactase enzymes studied, Maxilact® derived from S. lactis was found to be most suitable for the manufacture of hydrolyzed lactose yogurt by the simultaneous hydrolysis-fermentation procedure. In a consumer evaluation comparing lactase-supplemented to the equivalent unhydrolyzed yogurt, the hydrolyzed lactose product was significantly preferred ($P<.01$). Increased sweetness, decreased incubation time, and smoother body in lactose-hydrolyzed yogurts were also reported. In addition, less wheying-off was observed in the hydrolyzed lactose yogurts. This method was claimed to be more convenient and time saving than prehydrolyzing milk for culturing.

Technically, it is feasible to manufacture yogurt from lactose hydrolyzed milk. The process of hydrolysis can take place during overnight storage of cold milk or before heat treatment if the milk is tempered to the optimum temperature of the enzyme, e.g., 35 to 37°C. In either event, only slight disruption in factory routine occurs (103).

Other Applications of Lactase Enzyme

The use of β-galactosidase as a solution to the problems of lactose intolerance, whey utilization, lactose crystallization, and as a means for producing sweetener for the dairy and food industries has been well reviewed (39, 69, 93). Holsinger (39) evaluated lactose modified beverage milk for its physical and organoleptic properties and reported that hydrolyzing up to 60% of the lactose present in the milk resulted in the little change in consumer acceptance; hydrolyzing 90% of the lactose decreased the acceptance score. Milk with 30, 60, or 90% of its lactose converted to monosaccharides was equivalent in sweetness to a control milk containing .3, .6, or .9% added sucrose. This was because the hydrolytic end products from lactose, both glucose and galactose, are sweeter than lactose (33).

Cheddar cheese was made by Thompson and Brower (105) using milk with 65 to 80% of its lactose-hydrolyzed by lactase; and it was observed the hydrolyzed lactose cheddar had better flavor, with improved body and texture, than samples of cheese with no enzymatic

hydrolysis of lactose. The presence of free glucose as a readily available carbon source for Streptococcus lactis decreased the ripening period by 15 to 20 min because of the rapid acid development during setting.

Guy and Edmondson (32) prepared syrups by hydrolysis of lactose using lactase (Maxilact®) enzyme. The hydrolyzed lactose syrups were as sweet as sucrose syrups above 50% total solids. Differences in sweetness were small when lactose hydrolysis was increased from 75 to 90%.

Syrups prepared by heating hydrolyzed acid whey resulted in a golden colored, very sweet product for blending individually with other basic food materials to yield Swiss-type flavored yogurts, imitation maple syrups, and pudding (2). Syrups from hydrolyzed lactose, because of their sweetness at high solids, might find application in blending with high solids corn or sugar cane syrups, or use in high sugar baked goods. Because of their humectant properties as well as sweetness, they may find application in confections.

Use of lactose hydrolysis in ice cream ingredients to prevent sandiness, which arises from lactose crystallization, has been reported extensively (33, 41, 60, 93). Lactose hydrolysis prevents the formation of lactose crystals and hence, protein instability. Ice cream made experimentally using lactase-treated whey to supply 25% of the total serum solids showed possibilities of sucrose reduction because of the sweetening effect of the hydrolyzed lactose

(10, 47, 79). Organoleptic evaluations indicated that acceptable flavor and body could be achieved in these ice creams with 10% reduction in sucrose level. Beta-galactosidase seems to have a real potential in the development of frozen whole milk concentrate of good flavor and stability when reconstituted for the domestic market (93).

Kosikowski and Wierzbicki (54) used S. lactis lactase in treating raw and pasteurized whole milk and obtained 80% hydrolysis of the lactose in pasteurized milk and 75% in raw milk incubated at 4°C for 48 h with an enzyme concentration of 25 mg/liter. Flavor qualities other than sweetness in raw and pasteurized milks were not disturbed by lactase activity. However, when 100% lactose hydrolysis was attained, the samples possessed slight but noticeable chemical-like flavor. Adding very small amounts of sterilized lactase to milk or whey in sterile packages and allowing the hydrolysis to proceed for days, weeks, or months at room temperature, was reported by Dahlqvist et al. (16). Five mg of Maxilact® was sufficient for complete hydrolysis of lactose in 1 liter milk after 1 mo of incubation without any loss of biologically available lysine. However, after 3 to 5 mo there was a 9 to 13% loss and after 8 mo a 26% loss of lysine. It was suspected that lactose hydrolysis by this method might enable whey to be used in beverages.

Immobilization of Lactase

The purity, availability, and cost of β-galactosidase

become important economic considerations in any large scale lactose hydrolysis process. Although satisfactory hydrolysis is obtainable through addition of free enzymes, their one-time use appears uneconomical. Recent developments in enzyme immobilization permit continuous extended use of the bound enzymes and can reduce cost significantly (124).

Techniques for immobilization of lactase have been proposed by several investigators (26, 66, 76, 124). Moore (66) immobilized β-galactosidase from Aspergillus niger by coupling it chemically to controlled-pore silica beads and produced high quality glucose-galactose syrup with increased solubility, sweetness, and crystallization stability by hydrolyzing lactose of whey. Woychik and Wondolowski (124) reported fungal enzyme appeared more suited for use in immobilized systems than yeast or bacterial enzymes. The fungal enzyme from A. niger was bonded to glass beads and was used to hydrolyze acid whey. The bound enzyme had the same functional and stability properties as the free enzyme and retained approximately 75% of its original activity.

Okos et al. (76) reported β-galactosidase immobilized on phenol formaldehyde resin could provide a viable and economical method to commercially hydrolyze lactose in acid whey. The immobilized enzyme hydrolyzed lactose continuously at 40°C and pH 4.0 for over 120 days with no decrease in activity. Activity of the immobilized enzyme was similar to that of free enzyme. Weetal et al. (115) reported the shelf-life of A. niger β-galactosidase

bound to a porous glass to be 27.4 days at 40°C.

One potentially serious problem with immobilized lactase enzyme reactor systems is microbial growth in the reactor bed (80). Microbial contamination will lower the operational life of an enzyme reactor. Use of quaternary amines (200 ppm aqueous solution) to sanitize immobilized lactase enzyme systems without causing any loss in enzyme activity has been suggested.

Giacin et al. (26) reported immobilization of fungal β-galactosidase on collagen and utilization of collagen-bound lactase for hydrolysis of lactose in acid whey was quite feasible. Immobilized β-galactosidase will soon become an industrial catalyst and therefore a commodity of high economic potential (93).

Federal Standards of Identity for Yogurt

The first Federal Standards of Identity for Yogurt were published in 1981. According to Federal Standards (24) yogurt before the addition of bulky flavors should contain not less than 3.25% milk fat and not less than 8.25% milk solids-not-fat. Lowfat yogurt, before addition of bulky flavors, should contain not less than .5% milk fat nor more than 2% milk fat and not less than 8.25% milk solids-not-fat. Nonfat yogurt should contain less than .5% milk fat and not less than 8.25% milk solids-not-fat. The titrable acidity should not be less than .9% expressed as lactic acid. The food may be homogenized and shall be pasteurized or ultra-pasteurized prior to the addition of bacterial culture and

bulky flavoring material. To extend the shelf-life of the food, the yogurts may be heat-treated after culturing is completed, to destroy the viable microorganisms. However, the heat treatment after culturing may cause partial or complete loss of volatile flavors and destruction of some or all of the enzymes and culture organisms. Heating to inactivate the starter also inactivates lactase. The lactase, if not inactivated could be beneficial to lactose-intolerant consumers (98). Auxiliary labeling, "heat treated after culturing", is required if the yogurts are heat treated after culturing.

The subject of yogurt with bacteria versus yogurt without bacteria (pasteurized yogurt) has been discussed by Kroger (56), Kroger and Fram (58), and Speck and Geoffrion (98). There still exists controversy between the two views. The healthful aspect of living yogurt microorganism has been discussed by Speck (96), Shahani and Chandan (91), and Speck and Geoffrion (98). These and many other persons claim the yogurt should contain viable microorganisms for best health giving purposes.

The essential raw materials for yogurt as specified by Federal Standard, include cream, milk, partially skimmed milk, or skim milk, used alone or in combination. The optional ingredients allowed are concentrated skim milk, nonfat dry milk, buttermilk, whey, lactose, lactalbumins, lactoglobulins, and modified wheys. Natural or artificial flavoring substances are permitted. Stabilizers are permitted but are not named. No preservatives are

permitted. If reconstituted nonfat dry milk is used it places the product outside the standards for yogurt; it must be labeled as "reconstituted nonfat yogurt".

MATERIALS AND METHODS

Yogurt mixes were prepared from nonfat dry milk (NDM) with three levels of whey used in replacement for an equal amount of NDM, and three levels of lactose hydrolysis. Nine formulations of mixes were made to involve the different levels of lactose hydrolysis and replacement of NDM with sweet dry whey (Table 4). There were a total of three different formulations each with 12% nonfat dry milk; 9% NDM and 3% sweet dry whey; and 6% NDM and 6% sweet dry whey. Within each of the formulations, three different mixes were made: one with no hydrolysis; one with 50% of the lactose hydrolyzed; and one with 75% of the lactose-hydrolyzed.

TABLE 4. Types of yogurt formulae used.

Formula	Lactose hydrolysis		
	No hydrolysis	50% hydrolysis	75% hydrolysis
12:0[a]	1	1	1
9:3[b]	1	1	1
6:6[c]	1	1	1

[a]Yogurt made with 12% NDM.

[b]Yogurt made with 9% NDM and 3% dry whey.

[c]Yogurt made with 6% NDM and 6% dry whey.

Mix Formulations

One kilogram (2.2 lb) of each mix was prepared at one time. The batches with no whey solids were made with the following composition: 12% NDM[1], 2% anhydrous milk fat[2], 4% sucrose[3], and .5% stabilizer[4]. Three percent and 2% sucrose, respectively, were used in 50 and 75% lactose hydrolyzed batches; extra NDM was used to compensate the omitted sucrose. For other experimental batches, sweet dry whey[5] was used at 3% and 6% levels to replace an equivalent amount of NDM and the amount of sucrose was reduced in formulations with hydrolyzed lactose. Nine batches of plain yogurts were manufactured (Table 5) in one experimental series. Series were replicated five times.

The ingredients were calculated on a weight basis as shown in Appendix Table 1. First the required amount of NDM and sweet dry whey were weighed on a 1.5 kg basis. One and one-half kg of the mix was equivalent to 1305 ml, whereas, 1.0 kg of the mix was equivalent to 870 ml. All the mixes were based on 870 ml being

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[1]Spray dried, Grade A, nonfat dry milk. Land O'Lakes, Inc., Minneapolis, MN 55413.

[2]Land O'Lakes, Inc., Volga, SD 57071.

[3]"White Satin", fine granulated sugar. Amalgamated Sugar Company, Ogden, UT 84401.

[4]Stauffer Chemical Co., Food Ingredients Division, Milk Protein Group, Clawson, MI 48017.

[5]Sweet dry whey, Extra Grade. Land O'Lakes, Inc., Minneapolis, MN 55413.

TABLE 5. Formulation of different types of yogurt bases.

Composition	No hydrolysis	50% hydrolysis	75% hydrolysis
	(%)		
12:0			
NDM	12	13	14
Dry whey	0	0	0
Anhydrous milk fat	2	2	2
Sucrose	4	3	2
Stabilizer	.5	.5	.5
TOTAL	18.5	18.5	18.5
9:3			
NDM	9	10	11
Dry whey	3	3	3
Anhydrous milk fat	2	2	2
Sucrose	4	3	2
Stabilizer	.5	.5	.5
TOTAL	18.5	18.5	18.5
6:6			
NDM	6	7	8
Dry whey	6	6	6
Anhydrous milk fat	2	2	2
Sucrose	4	3	2
Stabilizer	.5	.5	.5
TOTAL	18.5	18.5	18.5

equivalent to 1 kg by weight. The rest of the ingredients were dissolved or suspended at the time of manufacturing of yogurt. The required amounts of NDM and sweet dry whey were added slowly to the required amount of distilled water in a 2 liter Erlenmeyer flask with simultaneous stirring with a magnetic bar to have a uniform mixture or slurry. Only 870 ml of the slurry was used for lactose hydrolysis while the remainder was kept refrigerated unhydrolyzed, to be used in blending to have the exact 50 or 75% lactose hydrolysis. All the nine batches were prepared in 1 day; lactose hydrolyzed NDM-whey slurry was used in six experimental batches.

Lactose Hydrolysis

Maxilact®,[1] L 2000, a dairy yeast lactase produced from Saccharomyces lactis, was used to hydrolyze the lactose in slurries of NDM and sweet dry whey. This enzyme hydrolyzes the β-D-galactoside linkage of lactose with an activity of 8000 ONPG/g (Orthonitrophenyl galactoside/g) and converts lactose into glucose and galactose. According to GB Fermentation Industries' Technical Bulletin on the enzyme Maxilact®, it is most effective between pH 6.6 and 7.0; the normal pH of milk, but it can be used to treat sweet dry whey. The enzyme is effective at all temperature ranges between 4 to 35°C. At a given enzyme dosage, the higher the

[1] GB Fermentation Industries Inc., 5550 77 Center Drive, Charlotte, NC 28224.

temperature the faster the hydrolysis, so at higher temperatures less enzyme will be required to attain the desired degree of hydrolysis in a given length of time. To avoid microbial spoilage, the mix should be pasteurized if the enzyme is to be used at higher temperatures of 30 to 35°C. In this study, incubation at lower temperature and longer time was selected to avoid double pasteurization of the mix.

The enzyme Maxilact® is considered to be a "generally regarded as safe" (GRAS) substance based on the following facts (4): 1) Petition for Affirmation of GRAS status filed by the Ad Hoc Enzyme Technical committee on April 11, 1973 (22); 2) This petition places carbohydrases from Saccharomyces species in the same category as the widely used food enzymes produced by Bacillus subtilis, Aspergillus niger, and Aspergillus oryzae (23).

One ml of the lactase enzyme was added per kilogram (870 ml) of the slurry and incubated at refrigeration temperature (4°C) for 16 h ± 1 h to achieve about 80% lactose hydrolysis. The lactase in the hydrolyzed mixes was inactivated by heating the mixes to 70°C (158°F) and holding at that temperature for 2 min. The amount of lactose present before and after the hydrolysis was determined by a colorimetric method (70). Once the initial degrees of lactose hydrolysis in the NDM and dry whey slurries was determined, unhydrolyzed NDM and dry whey slurries were blended with hydrolyzed slurries to attain the desired final percent of lactose hydrolysis in the mix such as 50% or 75%.

Culture Propagation

Hansens[1] freeze dried yogurt culture, Dri-Vac, Strain CHl, No. 5066, was used in this experiment. The organisms Streptococcus thermophilus and Lactobacillus bulgaricus were isolated and separately propagated. Elliker broth and plain agar were used to isolate S. thermophilus and Plate Count Agar was used for L. bulgaricus. About 50 mg of freeze dried culture was transferred aseptically to a sterilized reconstituted NDM (120 g/liter) medium. After incubation, the mixed culture was streaked onto solidified agar in a petri dish. Smears from the colonies that grew on the plates were Gram stained; then colonies were transferred to sterile culture media, which had been autoclaved at 121°C for 15 min. The isolated organisms were propagated separately, L. bulgaricus at 45°C and S. thermophilus at 37°C. For propagation, 1% inoculum from the mother culture was transferred to a fresh culture medium and incubated overnight for 15 h. The cultures were transferred daily during yogurt manufacturing and on alternate days for the rest of the period. Freshly grown cultures were used each time for manufacturing.

Manufacturing of Yogurt

After adjusting the NDM-whey slurries to the desired levels of lactose hydrolysis, the proper amount of sucrose and stabilizer

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[1]Chr. Hansens Laboratory, Inc., 9015 West Maple St., Milwaukee, WI 53214.

FIGURE 1. Flow diagram of yogurt manufacture.

NONFAT DRY MILK

AND SWEET DRY WHEY SUSPENSIONS

Non hydrolyzed mixes
↓
Stir sucrose and stabilizer into the mixes
↓
Heat to 45°C and add melted anhydrous milk fat
↓
Homogenize at 65°with a manual laboratory homogenizer
↓
Pasteurize at 70°C for 30 min
↓
Cool to 45°C
↓
Inoculate with 4.5% cultures
↓
Dispense into 500 ml containers
↓
Incubate at 42°C for 6 h
↓
Check TA and pH
↓
Refrigerate
↓
Organoleptic evaluations and compositional analyses

Hydrolyzed mixes
↓
Add 1 ml of enzyme and incubate overnight at 4°C
↓
Inactivate the enzyme at 70°C for 2 min
↓
Check the extent of hydrolysis
↓
Adjust the slurries to the desired level of lactose hydrolysis by blending with unhydrolyzed slurry
↓
Stir sucrose and stabilizer into slurries
↓
Heat to 45°C and add melted anhydrous milk fat
↓
Homogenize at 65°C with a manual laboratory homogenizer
↓
Pasteurize at 70°C for 30 min
↓
Cool to 45°C
↓
Inoculate with 4.5% cultures
↓
Dispense into 500 ml containers
↓
Incubate at 42°C for 6 h
↓
Check TA and pH
↓
Refrigerate
↓
Organoleptic evaluations and compositional analyses

were stirred into the bases in a 2 liter Erlenmeyer flask. Melted anyhdrous milk fat was added at 45°C. The mix was homogenized in a hand powered homogenizer at 65°C and pasteurized at 70°C for 30 min. The mix was cooled to 45°C and fresh cultures of L. bulgaricus and S. thermophilus were added at the rate of 4.5% of the mix weight and the mixes were stirred well to have uniform distribution of culture organisms. The mixes were dispensed into 500 ml plastic containers and incubated at 42°C for 6 h. The pH was determined every hour for 6 h from the same 500 ml carton; and final titrable acidity expressed as percent lactic acid, was measured. The yogurts were then carefully transferred to a refrigerator. Three batches of yogurts were made each day for 3 days and on the fourth day sensory evaluations were made on all nine lots.

Sampling

Samples for the final analysis for lactose were taken after adjusting the NDM-whey slurries to the desired 50 and 75% lactose hydrolysis. Samples for compositional analyses were taken after homogenization but before culture inoculation. These samples were frozen in plastic sample bottles at about -10°C until analyzed. Yogurt samples, one 500 ml carton of each, were frozen (-18°C) for some compositional analyses.

Organoleptic Evaluation

The finished yogurts were organoleptically evaluated by

the dairy manufacturing faculty of South Dakota State University's Dairy Science Department. The panel consisted of three to five judges. All the nine samples were evaluated when fresh and after 1 wk of storage. The samples were numerically coded from 1 to 9 to prevent identification of samples. The samples were evaluated for flavor, and body and texture; and the scores were recorded on the American Dairy Science Association yogurt score card (Appendix Figure 1). The flavor scores were based on 10 points for perfect flavor and 5 points for perfect body and texture. Flavor, body and texture defects were indicated. The means of all scores from all the judges were compiled and coded onto computer analysis sheets.

Statistical Analysis

A 3 x 3 factorial design with three levels of lactose hydrolysis and three levels of whey solids was utilized in this experiment (99). The main effects of different levels of whey solids and different levels of hydrolysis were tested by the respective main effect and replication interaction.

Compositional Analyses

The water soluble nitrogen contents were determined on cultured samples to observe the extent of proteolysis caused by the culture organisms. The following analyses were made in duplicate on uncultured samples from each batch of yogurts. Fat content of yogurt mixes was determined by the Mojonnier method

(7, 8). Total solids were also determined by Mojonnier procedure (7, 8). Protein contents of all the samples were determined by the Association of Official Analytical Chemists Kjeldahl procedure (7). Lactose, before and after hydrolysis, was determined by the method described by Nickerson et al. (70). Non hydrolyzed samples were diluted to have the dilution factor of 50 as described. For hydrolyzed samples some modification was made to keep the dilution factor 12.5 instead of 25. In step 2, to 1 ml of filtrate, 1 ml of 1 N NaOH was added, as described under preparation of sample, and diluted to 10 ml. The third step was omitted. Color intensity was measured at 540 nm with a Bausch and Lomb Spectronic 20 spectrophotometer.

Titrable acidity expressed as percent lactic acid was measured by titrating 9 g of sample with .1 N NaOH to the phenolphthalein end point using a Nafis automatic acidity test bottle (1, 8). Using an Orion research digital ionalyzer Model 501, pH values were measured.

Water soluble nitrogen values were ascertained by the Vakaleris and Price (109) method on hydrolyzed samples with and without culture. The uncultured samples were analyzed to ascertain the extent of proteolysis caused by the enzyme. Since a less purified enzyme preparation was used to hydrolyze the lactose in this experiment than was used by Islam (43) and Whalen (120), some proteolysis was expected. The cultured sample was checked for water soluble nitrogen to ascertain the proteolysis caused by the

culture organisms. Twenty-five milliliters of sodium citrate-yogurt solution containing 1 g of the sample was used for soluble nitrogen determination by Kjeldahl method (7, 109).

Coliform and psychrotrophic bacteria count were determined by Standard Methods procedures (1). The viscosity of yogurt samples was determined by a Brookfield-Synchro-lactric viscometer. The samples were refrigerated and viscosity was measured immediately after taking the samples out of the refrigerator. Temperature was measured with a thermometer immediately after the consistency measurement. The Brookfield Viscometer, Model HBT, was operated at 2.5 rpm and employed a Helipath stand accessory and a "B" bar T type spindle. The Helipath stand allowed the spindle to be lowered slowly while rotating into the sample, eliminating the channeling effect normally experienced with highly viscous materials. A factor for the particular level of speed and spindle was taken from the descriptive bulletin on the Brookfield Viscometer and was multiplied by the reading observed. Results were recorded in centipoise.

RESULTS AND DISCUSSION

Results of analysis of average composition of yogurt mixes for total solids, fat, lactose, and protein of the nine different mixes at 0, 50, and 75% levels of lactose hydrolysis are presented in Table 6. The overall means at three levels of hydrolysis and standard deviations are also tabulated. Knowledge of composition of the product is important because of the legal requirements set

TABLE 6. Average[a] composition of yogurt bases with 0, 50, or 75% of lactose hydrolyzed.

Components	Percent of lactose hydrolysis	Ratio of NDM: dry whey in yogurt bases 12:0	9:3	6:6	Mean	SD[b]
		%				
Protein	0	4.64[c,f]	4.02[c,g]	3.43[c,h]	4.03	.518
	50	5.02[d,f]	4.47[d,g]	3.86[d,h]	4.45	.498
	75	5.40[e,f]	4.81[e,g]	4.21[e,h]	4.81	.513
Fat	0	2.04[c,f]	2.01[c,f]	1.99[c,f]	2.01	.067
	50	2.03[c,f]	2.00[c,f]	2.02[c,f]	2.02	.063
	75	2.04[c,f]	2.03[c,f]	2.01[c,f]	2.03	.073
Lactose	0	7.38[c,f]	7.75[c,g]	8.38[c,h]	7.84	.505
	50	8.01[d,f]	8.91[d,g]	9.85[d,h]	8.92	.787
	75	8.51[e,f]	9.39[e,g]	10.28[e,h]	9.39	.754
Total solids	0	18.51[c,f]	18.48[c,f]	18.54[c,f]	18.51	.120
	50	18.56[c,f]	18.60[c,f]	18.52[c,f]	18.56	.099
	75	18.56[c,f]	18.57[c,f]	18.46[c,f]	18.53	.153
Solids-not-fat	0	16.47[c,f]	16.47[c,f]	16.55[c,f]	16.50	....
	50	16.53[c,f]	16.60[c,f]	16.50[c,f]	16.54	....
	75	16.52[c,f]	16.54[c,f]	16.45[c,f]	16.50	....

[a]Average of five replicates.

[b]Standard deviation.

[c,d,e]Means in the same column with different superscripts differ ($P<.01$).

[f,g,h]Means in the same row with different superscripts differ ($P<.01$).

by the standard of identity of yogurt.

Total Solids

The analysis of variance of total solids (Appendix Table 2) showed non significant differences among the yogurt batches with different percentages of whey solids and lactose hydrolysis. It was intended to keep the total solids constant at 18.5 ± .25%; the actual total solids in the mixes ranged from 18.27 to 18.73%. These values were within the 15.04 to 31.45% total solids range reported in Pennsylvania (55). Some differences could have resulted from variations in technique for taking samples in hydrolyzed batches after adjusting to the required level of lactose hydrolysis. This sampling was not required in non hydrolyzed batches. Small standard deviations (.15 or less) revealed the uniformity of solids among batches of yogurt. Percentages of solids-not-fat were estimated by substracting the fat percentages from percentages of total solids.

Lactose Content and Extent of Hydrolysis

The amount of lactose in yogurt bases was determined before and after enzymatic hydrolysis. Results (Table 6 and Appendix Table 3) indicated that as the percentages of whey solids in the bases were increased, the amounts of lactose were increased ($P<.01$). Since the dry whey contained 71% lactose as compared to 51% in the nonfat dry milk (NDM), increases in lactose percentage in whey-containing batches were expected. Similarly in lactose-hydrolyzed batches, since extra NDM was incorporated in lieu of omitted sucrose, an increase in lactose percentage was predicted and, in fact, occurred.

TABLE 7. Average actual percent of lactose hydrolysis attained in different bases.

Desired degree of lactose hydrolysis	Actual degree of lactose hydrolysis	Difference
	%	
12:0[a]		
50% Hydrolysis	51.4	+1.4
75% Hydrolysis	78.2	+3.2
9:3[b]		
50% Hydrolysis	50.6	+ .6
75% Hydrolysis	77.7	+2.7
6:6[c]		
50% Hydrolysis	52.4	+2.4
75% Hydrolysis	77.5	+2.5

[a,b,c]Yogurt bases containing 12:0, 9:3, or 6:6% NDM: dry whey, respectively.

Table 7 shows the average final adjusted lactose hydrolysis percentages in different lactose-hydrolyzed mixes. The average variations were within the range of +3.2% of the desired hydrolysis levels. Individual variations were within the range of ± 5% of the desired hydrolysis levels. The reasons for variation may have been in calculations, experimental errors, or instrumental errors. Analysis of variance of actual level of hydrolysis (Appendix Table 4) showed differences in levels of hydrolysis were significant ($P<.01$).

Fat

There were no important differences ($P<.05$) in fat percentage among lots. The fat percentages in the mixes varied from 1.92 to 2.11% (Table 6). These were legal for low fat yogurt according to Federal standards of identity for yogurt. These values were also within the range of .75 to 5.41% fat range reported in Pennsylvania (55) and .9 to 3.6% fat range reported in Canadian study cited by Kroger and Weaver (59).

Protein

The protein percentages were decreased as the percent of whey solids in formulations was increased, since dry whey contains less protein than NDM. The average protein at 0% hydrolysis level was 4.64% for lots with no whey solids, 4.02% for lots containing 3% whey solids, and 3.43% for lots with 6% dry whey. The protein percentages increased significantly in lactose-hydrolyzed batches because as the percent of lactose hydrolysis was increased, the level of sucrose was decreased and NDM was added instead of the

omitted sucrose. There was, of course, more protein in 75% lactose-hydrolyzed batches as compared to 50% lactose-hydrolyzed batches, since there was less sucrose and more NDM in the formula. The average protein content was 5.40% for batches with no whey solids and 75% hydrolysis of lactose as compared to 5.02% with 50% lactose hydrolysis (Table 6). The protein contents were within the 3.09 to 5.39% protein range found in commercial yogurts as reported in Pennsylvania studies (55, 59).

The analysis of variance for protein percentages is presented in Appendix Table 5. Differences in protein percentages among the lactose-hydrolyzed batches and whey solids containing batches were significant ($P<.01$).

Titrable Acidity and pH

The titrable acidities, expressed as percent lactic acid, are recorded in Table 8; while the analysis of variance of titrable acidities is shown in Appendix Table 6. A titrable acidity of 1% was desirable. All the batches contained 1% or more titrable acidity. The titrable acidities decreased as the level of whey solids in formulations increased. The yogurts containing 3% whey solids contained less acid ($P<.01$) than did those with no whey solids; and those with 6% whey solids content contained less acid ($P<.01$) than yogurts containing 3% whey solids or no whey solids.

TABLE 8. Titrable acidities[a], as percent lactic acid, of yogurts manufactured by three formulations with 0, 50, or 75% hydrolysis of lactose.

Desired percentage of lactose hydrolysis	Ratio of NDM: dry whey in yogurt bases			Mean	SD[b]
	12:0	9:3	6:6		
	% acidities				
0	1.18[c,f]	1.08[c,g]	1.00[c,h]	1.09	.078
50	1.26[d,f]	1.17[d,g]	1.12[d,g]	1.18	.088
75	1.32[e,f]	1.23[e,g]	1.16[d,h]	1.24	.099

[a]Values are means of five replications.

[b]Standard deviation.

[c,d,e]Means in the same column with different superscripts differ ($P<.01$).

[f,g,h]Means in the same row with different superscripts differ ($P<.01$).

This was because the titrable acidity contributed by casein is much higher than that from other components in milk such as lactalbumin, phosphates, or citrates; and whey solids lack casein. Moreover, NDM has more than two and one-half times as much protein as dry whey.

As the percentage of lactose hydrolysis was increased, the percent titrable acidity which developed also increased. The 50% lactose-hydrolyzed batches produced more titrable acidity ($P<.01$) than the yogurts with no lactose-hydrolyzed, and 75% lactose-hydrolyzed batches produced more acidity ($P<.01$) than 50% lactose-hydrolyzed or lots with no hydrolyzed lactose. Apparently, the readily available monosaccharides, resulting from hydrolysis of lactose, served for accelerated fermentation by yogurt culture organisms. The culture organisms seemed more active at 50% lactose hydrolysis than at 75% lactose hydrolysis. This result was comparable to findings of Tamime (101, 102). Conversely, a reduction in incubation period could be expected because of the rapid fermentation.

The pH values determined on the yogurts are presented in Table 9, and analysis of variance of pH values are shown in Appendix Table 7. A pH value of 4.30 to 4.40 was desirable. pH values of 4.36 or less were achieved in all the batches. As the level of whey solids was increased, increases in pH values were noticed. The pH values of 3% whey containing batches were higher ($P<.01$) than lots containing no whey solids, and those with 6% whey solids content had higher ($P<.01$) pH values than lots with 3% whey solids

TABLE 9. pH values[a] of yogurts manufactured from three formulations with 0, 50, or 75% hydrolysis of lactose.

Desired percentage of hydrolysis	Ratio of NDM: dry whey in yogurt bases			Mean	SD[b]
	12:0	9:3	6:6		
	pH readings				
0	4.23[c,f]	4.30[c,g]	4.36[c,h]	4.30	.059
50	4.17[d,f]	4.23[d,g]	4.27[d,h]	4.22	.055
75	4.14[e,f]	4.20[e,g]	4.24[e,h]	4.19	.059

[a]Values are means of five replicates.

[b]Standard deviation.

[c,d,e]Values in the same column with different superscripts differ ($P<.01$).

[f,g,h]Values in the same row with different superscripts differ ($P<.01$).

or no whey solids. Again, this was because of less protein and of casein in whey solids. As the percentages of lactose hydrolysis were increased, the pH values were decreased. The 50% lactose-hydrolyzed batches had lower ($P<.01$) pH values than yogurts with no lactose hydrolyzed, and 75% lactose-hydrolyzed batches had lower ($P<.01$) pH values than 50% lactose-hydrolyzed or lots with no hydrolyzed lactose. Again, likely this was because of the rapid fermentation of resultant simple sugars by yogurt microorganisms.

Water Soluble Nitrogen

Water soluble nitrogen contents of yogurts were determined in uncultured and cultured samples to determine the extent of proteolysis caused by the lactase enzyme and yogurt culture organisms, respectively. The water soluble nitrogen values are tabulated in Table 10, and the analysis of variance is presented in Appendix Table 8. The amount of water soluble nitrogen in the yogurts differed directly ($P<.01$) with the amount of whey solids in the yogurt bases. This likely was because the proteins in whey, if undenatured, are water soluble, whereas, NDM contains casein which is virtually insoluble in water. Significantly higher ($P<.01$) water soluble nitrogen values in lactose-hydrolyzed batches could have been due to proteolytic activity of the lactase enzyme used in this study. A relatively less expensive grade of lactase enzyme (Maxilact L 2000; $35/kg) was used in these trials. It was not as highly purified as some preparations and was found to

TABLE 10. Average water soluble nitrogen contents[a] of uncultured and cultured yogurts.

Samples	Desired percentage of lactose hydrolysis	Ratio of NDM: dry whey in yogurt bases			Mean	SD[b]
		12:0	9:3	6:6		
		% water soluble nitrogen				
Uncultured	0	.191^{c,f}	.207^{c,g}	.229^{c,h}	.209	.019
	50	.203^{d,f}	.225^{d,g}	.253^{d,h}	.227	.023
	75	.218^{e,f}	.245^{e,g}	.265^{e,h}	.243	.026
Cultured	0	.169^{i,l}	.182^{i,l}	.204^{i,m}	.185	.030
	50	.187^{j,l}	.192^{i,l}	.215^{i,m}	.198	.025
	75	.203^{k,l}	.206^{j,l}	.235^{j,m}	.215	.025

[a]Values are means of five replicates.

[b]Standard deviation.

^{c,d,e,i,j,k}Means in the same column for a given series (cultured/uncultured) with different superscripts differ ($P<.01$).

^{f,g,h,l,m}Means in the same row with different superscripts differ ($P<.01$).

contain some proteases which would break down the peptide bonds of protein, resulting in water soluble peptides and amino acids. Tamime and Deeth (103) mentioned the use of a lactase enzyme resulted in enhanced proteolysis which may have been partially due to the presence of proteases in the yeast-derived β-D-galactosidase preparations.

The proteolysis caused by culture organisms was also significantly higher ($P<.01$) in whey-containing and lactose-hydrolyzed batches (Table 10 and Appendix Table 9). The yogurts containing 6% whey solids developed significantly higher water soluble nitrogen contents during fermentation than those with 3% whey solids or no whey solids. Similarly, the 75% lactose-hydrolyzed batches had higher soluble nitrogen values after fermentation than batches with 50% or no lactose hydrolysis. The results of proteolytic activity of culture organisms were comparable to those observed by Singh and Sharma (95) and Singh et al. (94).

Some of the yogurts were criticized for being slightly bitter after 1 wk of storage and definitely bitter after 3 wk of storage. This could have been because of the proteolytic activity by L. bulgaricus during storage. An adverse effect of proteolysis in dairy products is release of bitter peptides. In yogurt, this has been attributed (103) to proteolysis by L. bulgaricus during storage. Yogurts incubated at 44°C are less likely to be bitter than those produced at 38°C (103). Bitterness was also noticed in some of the fresh yogurt samples as noted earlier, it was

concluded that this bitterness was due to proteolytic activity of the lactase enzyme preparation.

Bacterial Count

All the samples had <10/g coliform and psychrotrophic bacterial counts, in accordance with Federal standards for yogurt. This was well, for coliforms are detrimental to the dairy industry. They are Gram-negative, non-spore forming bacteria which ferment lactose to acid and gas and give milk and its products a very undesirable flavor (8).

Pasteurization destroys coliform bacteria. The results indicated the yogurt mixes were well pasteurized. Moreover, the dry whey and NDM used had been heat treated during drying, so these bacteria were not expected to be present in large numbers.

The psychrotrophs are Gram-negative, non-spore forming rods. The majority of these psychrotrophic species are said to be inert; that is, their metabolic products do not produce marked changes in milk. However, there are many species which are either proteolytic or lipolytic. These may ruin the market quality of milk through the production of undesirable flavors. While pasteurization destroys psychrotrophs, it can not overcome the off-flavors. Since the samples had <10/g psychrotrophs, not much proteolysis was expected in the experimental yogurts from these bacteria.

Organoleptic Evaluations

Table 11 shows flavor scores of fresh and 1 wk old yogurts, as assigned by the Dairy Science Department panel. The flavor scores were based on a hedonic scale with 10 being a perfect score. There were no significant differences between scores of the batches containing 0 or 3% whey solids and with no lactose hydrolysis. However, yogurts with 6% whey solids were scored significantly lower. There were small non significant differences in scores among hydrolyzed lactose lots (Appendix Table 10). The interactions of effects of whey solids and lactose hydrolysis upon flavor scores of batches is represented graphically in Figure 2. The hydrolyzed batches with 6% whey solids scored significantly higher on flavor than did unhydrolyzed or 50% lactose-hydrolyzed yogurts with the same formulation. Analysis of variance of scores of 1 wk old yogurts also showed no significant differences of scores assigned unhydrolyzed lots containing 0 or 3% whey solids (Appendix Table 11). There were no differences in flavor scores among 50% and 75% lactose-hydrolyzed batches. However, the flavor scores of batches with no whey solids were higher than either 50 or 75% lactose hydrolyzed batches.

In the sensory evaluations, the most common flavor defects noticed were "lack of fine flavor". Some of the batches were criticized as having "cooked" flavor. This may have been caused by overheating of the mixes during pasteurization in the Erlenmeyer flasks. Since the NDM and dry whey had already been heat-treated

TABLE 11. Flavor scores[a,b] assigned by dairy faculty panel to fresh and 1 wk old yogurts which were manufactured by three formulations with 0, 50, or 75% hydrolysis of lactose.

Age of samples	Percent of lactose hydrolysis	Ratio of NDM: dry whey in yogurt bases			Mean	SD[c]
		12:0	9:3	6:6		
		Flavor scores[b]				
Fresh	0	8.88[d,f]	8.63[d,f]	7.63[d,g]	8.38	.732
	50	8.38[d,f]	8.25[d,f]	7.99[d,e,g]	8.21	.281
	75	8.01[d,f]	8.12[d,f]	8.29[e,g]	8.14	.515
1 wk old	0	8.95[d,f]	8.25[d,f]	7.47[d,g]	8.22	.859
	50	7.62[e,f]	7.75[e,f]	6.86[e,g]	7.41	1.176
	75	7.26[e,f]	7.10[e,f]	7.36[e,g]	7.24	1.247

[a]Values are means of five replicates.

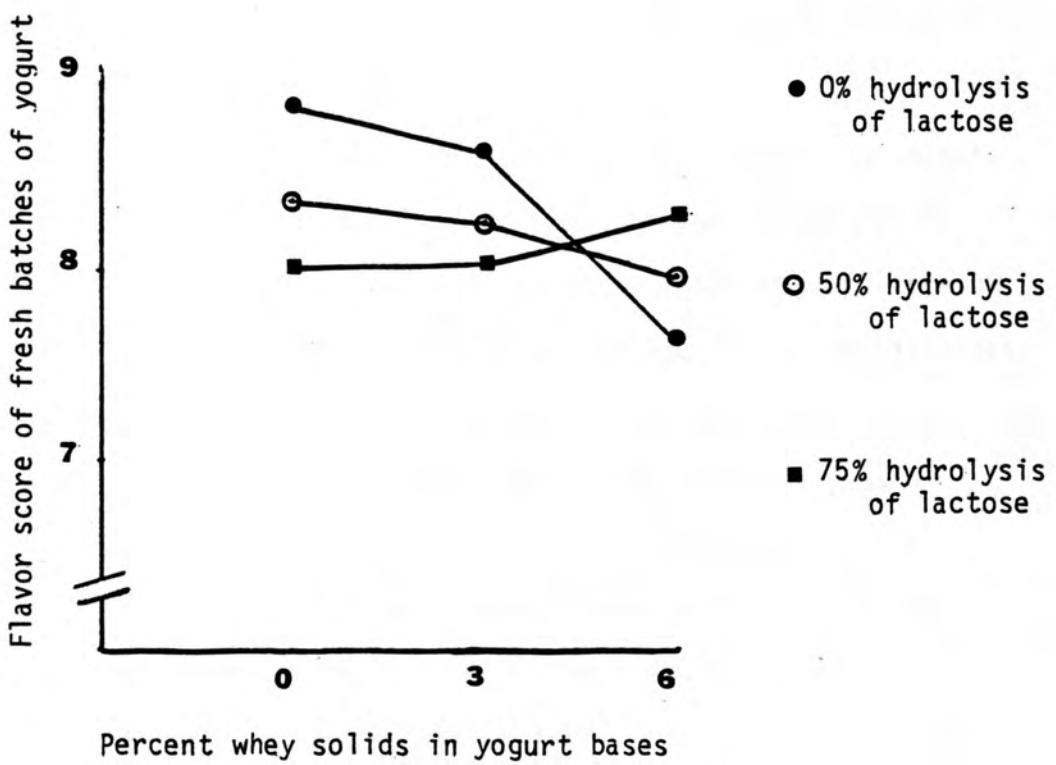
[b]Based on a hedonic scale of 1 to 10 with 10 as perfect score.

[c]Standard deviation.

[d,e]Means in the same column for fresh or week old samples with different superscripts differ ($P<.05$).

[f,g]Means in the same row with different superscripts differ ($P<.05$).

FIGURE 2, Flavor scores of yogurts as affected by interactions of different levels of lactose hydrolysis and different levels of whey solids.


Flavor score of fresh batches of yogurt
9
8
7
0
3
6
Percent whey solids in yogurt bases
● 0% hydrolysis of lactose
⊙ 50% hydrolysis of lactose
■ 75% hydrolysis of lactose

during drying operations, even slight overheating may have caused this problem; or the flavor simply may have resulted from those prior heat treatments. Some of the batches were cited as being "too sweet", which indicated further reduction of sucrose level could have been achieved in lots with portions of the lactose hydrolyzed. Some of the batches were also criticized for "unnatural flavoring". This flavor had been noted in ice cream which Islam (43) made with 50% or 75% hydrolysis of lactose in milk derived ingredients. Bitterness was also noticed in some of the samples. This could have been from the proteolytic activity of culture organism and/or lactase enzyme.

"Lack of flavoring" and "lack of fine flavoring" defects were also noticed in yogurts after 1 wk storage. Some of the batches were also criticized for unnatural flavoring and foreign flavor. In yogurt samples after 3 wk storage, definite bitterness was noticed, likely due to proteolysis caused by the lactase enzyme preparation and/or culture organisms. After storage some of the samples were also criticized for lack of freshness.

The average body and texture scores of fresh and stored yogurts are presented in Table 12. The analysis of variance of average body and texture scores of fresh yogurt samples is shown in Appendix Table 12. There were no differences ($P<.05$) among the batches containing no whey or 3% whey solids. Analysis of interactions at different levels of whey solids content and lactose hydrolysis (Figure 3) showed significant increase in scores of

TABLE 12. Average body and texture scores[a,b] of fresh and 1 wk old yogurts manufactured from three formulations with 0, 50, or 75% hydrolysis of lactose.

Age of samples	Percent of lactose hydrolysis	Ratio of NDM: dry whey in yogurt bases			Mean	SD[c]
		12:0	9:3	6:6		
		scores[b]				
Fresh	0	4.38[d,g]	4.20[d,g]	2.82[d,h]	3.80	.714
	50	4.44[d,g]	4.32[d,g]	3.88[e,g]	4.21	.784
	75	4.31[d,g]	4.26[d,g]	4.43[f,g]	4.33	.512
1 wk old	0	4.05[d,g]	5.25[d,g]	3.60[d,h]	3.97	.550
	50	4.05[d,g]	4.45[d,g]	3.75[d,h]	4.08	.561
	75	4.00[d,g]	4.55[d,h]	4.50[e,h]	4.35	.565

[a]Values are means of five replicates.

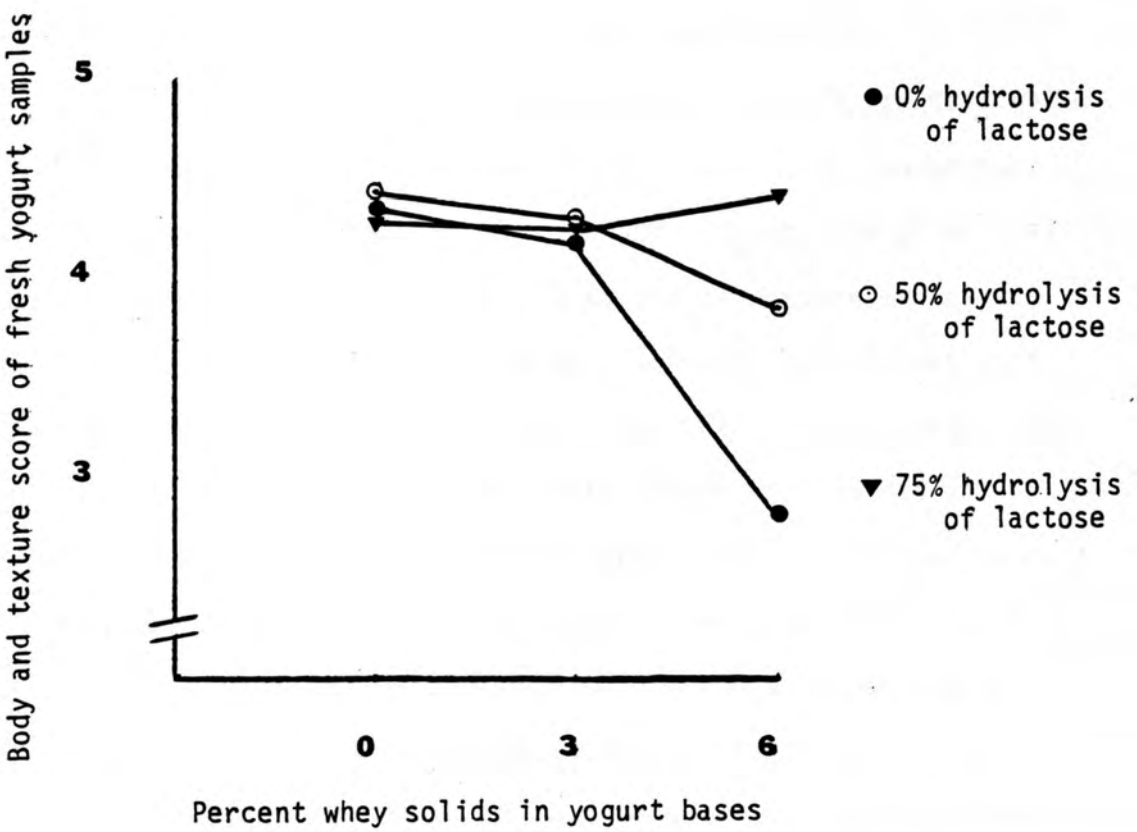
[b]Based on hedonic score of 1 to 5, with 5 as perfect score.

[c]Standard deviation.

[d,e,f]Means in the same column for a given series with different superscripts differ ($P<.05$ for fresh samples, $P<.01$ for 1 wk old samples).

[g,h]Means in the same row with different superscripts differ ($P<.05$ for fresh and $P<.01$ for 1 wk old samples).

FIGURE 3. Body and texture scores of fresh yogurt samples as affected by interactions of different levels of NDM replacement with dry whey and different levels of lactose hydrolysis.


Body and texture score of fresh yogurt samples
5
4
3
0
3
6
Percent whey solids in yogurt bases
● 0% hydrolysis of lactose
⊙ 50% hydrolysis of lactose
▼ 75% hydrolysis of lactose

yogurts containing 6% whey solids and with 75% of the lactose-hydrolyzed. This may have been because of extra NDM added in lieu of omitted sucrose.

In general, the batches without dry whey were criticized as being too firm, and batches containing 6% whey solids were cited for being too weak. It was concluded the batches containing 3% whey solids were suitable as having a spoonable type body. At 6% whey solids content, the body might have been improved by increasing the amount of stabilizer and would have been suitable for pourable type body. No "wheying-off" defect was reported.

The viscosity values are presented (Table 13) in centipoise units. The viscosities decreased as the whey solids contents of yogurt bases were increased. The analysis of variance of viscosities of yogurts (Appendix Table 13) showed significantly lower viscosities in batches containing whey solids. The batches with 3% dry whey content had lower viscosities than batches containing no whey solids; and yogurts containing 6% whey solids had still lower viscosities than those with 3% whey solids. This was in part because casein contributes much viscosity and whey lacks casein. More importantly, whey solids contributed much soluble lactose which lent fluidity. The hydrolyzed batches had significantly higher viscosities ($P<.05$). This was because extra NDM was added to keep total solids percentages the same in formulations having less sucrose as the percentage of lactose hydrolysis was increased.

TABLE 13. Average viscosities of yogurts[a] manufactured by three formulations and with 0, 50, or 75% of the lactose hydrolyzed.

Desired percentage of hydrolysis	Ratio of NDM: dry whey in yogurt bases			Mean	SD[b]
	12:0	9:3	6:6		
	CP				
0	46,400[c,f]	25,600[c,g]	20,800[c,g]	30,933.33	13,219.78
50	64,000[d,f]	44,000[d,g]	33,600[d,h]	47,200.00	13,865.64
75	72,000[e,f]	49,600[d,g]	44,800[e,g]	55,466.67	13,063.95

[a]Values are average of two replicates.

[b]Standard deviation.

[c,d,e]Means in the same column with different superscripts differ ($P<.01$ for ratio of NDM: whey; $P<.05$ for percent of lactose hydrolysis).

[f,g,h]Means in the same row with different superscripts differ ($P<.01$ for NDM: whey ratio; $P<.05$ for percent lactose hydrolysis).

Cost Analysis

The costs per kilogram of yogurt are presented in Table 14. The approximate costs are computed on the ingredients' prices given in Dairy Record (42). The cost of anhydrous milkfat, stabilizer, and distilled water were kept constant for all formulations. The cost variation was due primarily to lactase enzyme and diverse amounts of NDM, dry whey, and sucrose.

It is obvious from Table 14 that in hydrolyzed lactose yogurts with only NDM as the milk serum solids source, there was negative economic advantage because of the extra cost of enzyme and NDM which was more expensive than the omitted sucrose. As the whey solids were increased, the savings were also increased. With 3% whey solids there was an economic advantage of about 5¢ per kilogram of mix when no lactose was hydrolyzed. The cost advantage was from replacing NDM with less expensive dry whey. Cost savings dropped sharply as the mixes were treated to hydrolyze lactose. The effect was progressive, at 75% lactose hydrolysis the cost advantage was less than at 50% lactose hydrolysis because of adding more NDM in place of sucrose, for NDM costs four times as much, and the cost of more enzyme needed to hydrolyze available lactose. Much higher savings could be made by substituting dry whey in lieu of omitted sucrose, and obviously this should have been tried. From the economic and yogurt flavor viewpoints, yogurts made with 3% dry whey were more feasible; and hydrolysis of 50% of the lactose present was more practical than 75% lactose hydrolysis, even

TABLE 14. Costs[a] of unhydrolyzed and hydrolyzed lactose yogurts.

Formula	Cost per kg of yogurt	Cost advantage per kg of yogurt
	$	
12:0[b]		
0% hydrolysis	.527	.00
50% hydrolysis	.540	-.013
75% hydrolysis	.555	-.028
9:3[c]		
0% hydrolysis	.476	+.051
50% hydrolysis	.489	+.038
75% hydrolysis	.504	+.023
6:6[d]		
0% hydrolysis	.427	+.100
50% hydrolysis	.441	+.086
75% hydrolysis	.456	+.071

[a]Source: (42).

[b,c,d]Yogurt bases containing 12:0, 9:3, or 6:6% NDM: dry whey, respectively.

though yogurts with the greater hydrolysis scored well on flavor.

SUMMARY

The objectives of this project were to determine the acceptability of yogurt made with reconstituted nonfat dry milk or a blend of nonfat dry milk and sweet dry whey as the source of milk solids-not-fat, with or without hydrolysis of a substantial portion of the lactose present in the substrate. It was found to be feasible to manufacture yogurt with reconstituted NDM and with 25% of the NDM replaced with sweet dry whey. There were no adverse effects on flavor with these substrates. It was also found to be possible to manufacture lactose-hydrolyzed yogurt with only slight disruption in factory routine. The process of hydrolysis was accomplished during overnight storage of the yogurt mix at refrigeration temperature. Heat treatment to inactivate lactase and preclude further lactose hydrolysis may have been a contributing cause of cooked flavor in the experimental yogurts. With experience the total time/temperature of heating could be adjusted to obviate this problem. Fifty percent replacement of NDM with dry whey was a questionable practice because of the resultant lower flavor scores and the effect of whey on body and texture.

Specific Conclusions

1. Yogurts without enzymatic hydrolysis of lactose were preferred slightly over the lactose-hydrolyzed batches except the one with 6% whey solids. There was less preference for 75%

lactose-hydrolyzed batches as compared to unhydrolyzed or 50% lactose-hydrolyzed batches. No significant differences in flavor preferences were found among the lactose-hydrolyzed batches.

The 75% lactose-hydrolyzed batches with 6% whey solids were preferred over unhydrolyzed yogurts or 50% lactose-hydrolyzed batches with 6% whey solids. So, at higher levels of lactose hydrolysis, a satisfactory product could be achieved with 6% whey solids in the yogurt bases.

2. The lactose-hydrolyzed batches produced significantly higher titrable acidity, expressed as percent lactic acid. Conversely, a reduction in time to achieve coagulation could be expected. Yogurts containing whey solids produced less acidity; those with 3% whey solids had lower acidity values than those without whey solids; and yogurts with 6% whey solids had lower acidity than those with 3% whey solids. However, the lactic acid content of yogurts containing 6% whey solids was still above the .9% legal minimum titrable acidity.

3. Some of the yogurts were criticized for being too sweet, indicating the possibility of further reduction of sucrose levels in yogurts with a substantial portion of their lactose content hydrolyzed. This was in agreement with previous studies by Dariani et al. (17) and Hilgendorf (38) on hydrolyzed lactose yogurts by simultaneous hydrolysis-fermentation method. Hydrolyzed lactose yogurts were reported as being sweeter than non hydrolyzed ones.

4. Yogurts composed in part of whey solids contained significantly less protein. As the amount of whey solids was increased

in formulations, protein percentage was decreased. However, protein contents of yogurts with 6% whey solids were still within the range of protein values found in commercial yogurts (55, 59).

5. Viscosities of yogurts which contained whey solids were significantly lower. Viscosity in yogurts is largely contributed by casein, and whey solids have no casein. Lower viscosities in whey-containing batches were desirable for pourable yogurt.

6. At 6% whey solids level, the bodies of yogurts were weak, while in yogurts with no whey solids the body was too firm. Yogurts with 3% whey solids had pourable type of consistency, which was being sought in this research. In commercial practice, the stabilizer level could be adjusted to achieve the particular type of body sought.

7. Water soluble nitrogen contents were significantly higher in yogurts containing whey solids and in lactose-hydrolyzed batches. A significantly higher soluble nitrogen in lactose-hydrolyzed batches indicated proteolytic activity of the yeast derived lactase preparation used in these studies.

8. Proteolytic activity by the culture organisms was also noticed.

9. Bitterness in some of the yogurts could have been partially due to release of proteoses and peptides via proteolytic activity of culture organisms and impurities in the lactase enzyme preparation.

10. Approximate cost analysis showed savings resulted from including whey solids in the yogurt formulations. Fifty percent lactose hydrolysis was more reasonable than 75% lactose hydrolysis from an economic viewpoint.

11. The results indicated that reduction of tendency to lactose crystallization when lactose was hydrolyzed permitted usage of relatively high whey solids contents in yogurts with satisfactory flavor and without increasing the problems of lactose-intolerance in lactose sensitive persons. Substitution of dry whey for more expensive NDM is an approach to savings in cost. The product with NDM did not comply with the Standards of Identity for yogurt in the USA but may be a good salable product for Nepal and other developing countries.

12. The substitution of dry whey in lieu of omitted sucrose as well as other levels of replacement of NDM with dry whey and/or modified whey products should be studied further. A previous study at this university by Whalen (120) indicated use of equal amounts of NDM and reconstructed milk products (WM-34 and WP-34) to fortify 2% low fat milk for making yogurt resulted in no detectable flavor differences among the batches fortified with 4% NDM and those having 50% NDM replaced with WM-34 or WP-34. Complete replacement of NDM with reconstructed milk products was a questionable practice. Although a highly purified lactase enzyme preparation (Maxilact® L x 5000) was used in the trials, non hydrolyzed yogurts were preferred over hydrolyzed lactose ones.

REFERENCES

1 American Public Health Association. 1978. Standard methods for the examination of dairy products. 14th ed. Am. Publ. Health Assoc., Washington, DC.

2 Anonymous. 1975. Some new uses for lactose in the light of the sugar shortage. Milk Ind. 76(4):15.

3 Anonymous. 1979. Using modified whey in dairy products. Dairy Ice Cream Field 162(4):82 I.

4 Anonymous. 1981. Regulatory status of Maxilact^{R}. Information bulletin. GB Fermentation Industries Inc., PO Box 241068, Charlotte, NC.

5 Arbuckle, W. S. 1972. Page 80 in Ice Cream. 2nd ed. The AVI Publ. Co., Westport, CT.

6 Arbuckle, W. S. 1979. Whey solids in frozen dessert formulations. Am. Dairy Rev. 41(12):50 D.

7 Association of Official Analytical Chemists. 1980. Official methods of analysis. 13th ed. Assoc. Offic. Anal. Chem., Washington, DC.

8 Atherton, H. V., and J. A. Newlander. 1977. Chemistry and testing of dairy products. 4th ed. AVI Publ. Co., Westport, CT.

9 Becker, F. 1971. Yogurt and culture production using "instant" dried skim milk. Ost. Milchw. 26(16):297. Cited from Dairy Sci. Abstr. 33:884.

10 Bills, D. D., C. S. Yang, M. E. Morgan, and F. W. Bodyfelt. 1972. Effect of sucrose on the production of acetaldehyde and acids by yogurt culture bacteria. J. Dairy Sci. 55:1570.

11 Borglum, G. B., and M. Z. Sternberg. 1972. Properties of a fungal lactase. J. Food Sci. 37(4):619.

12 Campbell, J. R., and R. T. Marshall. 1975. Page 607 in The science of providing milk for man. McGraw-Hill Book Company, Inc., New York, NY.

13 Chambers, J. V. 1979. Culture and processing techniques important to the manufacture of good quality yogurt. Cult. Dairy Prod. J. 14(2):28.

14 Cottenie, J. 1978. Yogurt processing in Europe. Cult. Dairy Prod. J. 13(4):6.

15 Cox, A. C. 1973. Whey powder. Food Processing Ind. 42(505):49.

16 Dahlqvist, A., N. G. Asp, A. Burvall, and H. Rausing. 1977. Hydrolysis of lactose in milk and whey with minute amounts of lactase. J. Dairy Res. 44:541.

17 Dariani, D. N., J. F. Frank, and M. Loewenstein. 1982. Manufacture of low lactose yogurt by simultaneous lactose hydrolysis and bacterial fermentation. Cult. Dairy Prod. J. 17(2):18.

18 DeHaast, J., P. M. Lategan, and J. C. Novello. 1979. Some aspects of yogurt quality: a review. S. African J. Dairy Technol. 11(1):11.

19 Delaney, R. A. M. 1981. Recent developments in the utilization of whey. Cult. Dairy Prod. J. 16(2):11.

20 Emaldi, G. C., C. Pompei, E. Carbone, and C. Peri. 1975. Composition and keeping quality of yogurt from milk, concentrated by ultrafiltration. Sciena e Technica Lattiero Casearia (1974) 25(2):33 (Dairy Sci. Abstr. 37:215).

21 Engel, W. G. 1973. The use of lactase to sweeten yogurt without increasing calories. Cult. Dairy Prod. J. 8(3):6.

22 Food and Drug Administration. 1973. Notice of filing of petition for affirmation of GRAS status. Fed. Reg. 38(70) (Sic. as cited in GB Fermentations bulletin).

23 Food and Drug Administration. 1973. Notice of filing of petition for affirmation of GRAS status. Fed. Reg. 38(112) (Sic. as cited in GB Fermentations bulletin).

24 Food and Drug Administration. 1981. Cultured and acidified milks, cultured and acidified buttermilk, yogurts, and eggnog; standards of identity. Fed. Reg. 46(20):9924.

25 Forsum, E., and L. Hambraeus. 1977. Nutritional and biochemical studies of whey products. J. Dairy Sci. 60:370.

26 Giacin, J. R., J. Jakubowski, J. G. Leeder, S. G. Gilbert, and D. H. Kleyn. 1974. Characterization of lactase immobilized on collagen: conversion of whey lactose by soluble and immobilized lactase. J. Food Sci. 39:751.

27 Glass, L., and T. I. Hedrick. 1977. Nutritional composition of sweet- and acid-type dry wheys. I. Major factors including amino acids. J. Dairy Sci. 60:185.

28 Glass, L., and T. I. Hedrick. 1977. Nutritional composition of sweet- and acid-type dry wheys. II. Vitamin, mineral, and calorie contents. J. Dairy Sci. 60:190.

29 Goodenough, E. R., and D. H. Kleyn. 1975. Qualitative and quantitative changes in the carbohydrates during the manufacture of yogurt. J. Dairy Sci. 29:45.

30 Granier, G. 1975. Process for manufacturing fermented milk products and products obtained. French Patent Application (1974) 2 224 096 (Dairy Sci. Abstr. 37:586).

31 Guy, E. J., and E. W. Bingham. 1978. Properties of β-galactosidase of Saccharomyces lactis in milk and milk products. J. Dairy Sci. 61:147.

32 Guy, E. J., and L. F. Edmondson. 1978. Preparation and properties of sirups made by hydrolysis of lactose. J. Dairy Sci. 61:542.

33 Guy, E. J., A. Tamsma, A. Kouston, and V. H. Holsinger. 1974. Lactase-treated milk provides base to develop products for lactose-intolerant populations. Food Prod. Develop. 8(8):50.

34 Gyuricsek, D. M., and M. P. Thompson. 1976. Hydrolyzed lactose cultured dairy products. II. Manufacture of yogurt, buttermilk, and cottage cheese. Cult. Dairy Prod. J. 11(3):12.

35 Harper, W. J., and C. W. Hall. 1981. Page 240 in Dairy technology and engineering. AVI Publ. Co., Inc., Westport, CT.

36 Hartman, G. H. 1975. The use of whey in the manufacture of yogurt. Cult. Dairy Prod. J. 10(2):6.

37 Hekmati, M., and R. L. Bradley, Jr. 1979. Utilization of acid whey in frozen yogurt. Cult. Dairy Prod. J. 14(2):6.

38 Hilgendorf, M. J. 1981. Optimization of fungal lactase levels in yogurt manufacturing. Cult. Dairy Prod. J. 16(1):5.

39 Holsinger, V. H. 1978. Application of lactose-modified milk and whey. Food Tech. 32(3):35.

40 Holsinger, V. H., L. P. Posati, and E. D. DeVilbiss. 1974. Whey beverages: a review. J. Dairy Sci. 57:849.

41 Holsinger, V. H., and N. E. Roberts. 1976. New products from lactose-hydrolyzed milk. Dairy Ice Cream Field 159(3):30.

42 Horner, C. 1982. Market situation. Dairy Record 83(5):79.

43 Islam, M. S. 1981. Hydrolyzed-lactose whey and nonfat dry milk in ice cream. M. S. thesis. Dairy Science Dept., South Dakota State University, Brookings.

44 Jelen, P., and H. Horbal. 1974. Utilization of cottage cheese whey in yogurt manufacture. J. Dairy Sci. 57:584.

45 Jenness, R., and S. Patton. 1959. Principles of dairy chemistry. John Wiley & Sons, Inc., New York.

46 Jespen, S. 1977. Membrane filtration in the manufacture of cultured milk products. Am. Dairy Rev. 39(1):29.

47 Keenan, T. W., and D. D. Bills. 1968. Metabolism of volatile compounds by lactic starter culture microorganisms: a review. J. Dairy Sci. 51:1561.

48 Kilara, A., and K. M. Shahani. 1976. Lactase activity of cultured and acidified dairy products. J. Dairy Sci. 59:2031.

49 Korner, H. 1973. Process for the production of yogurt. German Federal Patent Application (1972) 2 112 943 (Dairy Sci. Abstr. 35:449).

50 Kosikowski, F. V. 1976. Greater utilization of whey powder for human consumption and nutrition. J. Dairy Sci. 50:1343.

51 Kosikowski, F. V. 1977. Page 73 in Cheese and fermented milk foods. 2nd ed. Edwards Brothers, Inc., Ann Arbor, MI.

52 Kosikowski, F. V. 1979. Low lactose yogurts and milk beverages by ultrafiltration. J. Dairy Sci. 62:41.

53 Kosikowski, F. V. 1979. Whey utilization and whey products. J. Dairy Sci. 62:1149.

54 Kosikowski, F. V., and L. E. Wierzbicki. 1973. Lactose hydrolysis of raw and pasteurized milks by Saccharomyces lactis lactase. J. Dairy Sci. 56:146.

55 Kroger, M. 1973. Controlling the quality of yogurt. Dairy Ice Cream Field 156(1):38.

56 Kroger, M. 1975. How do you want your yogurt, with or without bacteria? A review of the controversy. Cult. Dairy Prod. J. 10(2):18.

57 Kroger, M. 1976. Quality of yogurt. J. Dairy Sci. 59:344.

58 Kroger, M., and S. R. Fram. 1975. Consumer attitudes toward yogurt. Food Technol. 29(11):52.

59 Kroger, M., and J. C. Weaver. 1973. Confusion about yogurt-compositional and otherwise. J. Milk Food Technol. 36(7):388.

60 Lang, F., and A. Lang. 1977. New developments in lactose hydrolysis and its use in dairy products. Milk Ind. 79(3):4.

61 Manus, L. J. 1973. Yogurt - how tart? Dairy Ice Cream Field 156(7):52.

62 Mathur, B. N., and K. M. Shahani. 1979. Use of total whey constituents for human food. J. Dairy Sci. 62:99.

63 McDonough, F. E. 1977. Whey solids utilization and salvage systems. Cult. Dairy Prod. J. 12(1):8.

64 Milk Facts. 1981. Milk Industry Foundation, Washington, DC.

65 Moon, N. J., and G. W. Reinbold. 1974. Selection of active and compatible starters for yogurt. Cult. Dairy Prod. J. 9(4):10.

66 Moore, K. 1980. Immobilized enzyme technology commercially hydrolyzes lactose. Food Prod. Develop. 14(1):50.

67 Morley, R. G. 1979. Potential of liquid yogurt. Cult. Dairy Prod. J. 14(4):30.

68 Morr, C. V. 1979. Functionality of whey protein products. New Zealand J. Dairy Sci. and Technol. 14(2):185.

69 Nickerson, T. A. 1978. Why use lactose and its derivatives in foods? Food Technol. 32(1):40.

70 Nickerson, T. A., I. F. Vujicic, and A. Y. Lin. 1976. Colorimetric estimation of lactose and its hydrolytic products. J. Dairy Sci. 59:386.

71 Nickerson, T. A., B. H. Webb, A. H. Johnson, and J. A. Alford. 1978. Page 300 in Fundamentals of dairy chemistry. 2nd ed. AVI Publ. Co., Inc., Westport, CT.

72 Nielsen, V. H. 1975. Replacement of nonfat milk solids with dry whey. Am. Dairy Rev. 37(2):22.

73 Nielsen, V. H. 1976. Use of whey solids in cultured milk products. Cult. Dairy Prod. J. 11(1):12.

74 Nielsen, M. A., S. T. Coulter, C. V. Morr, and J. R. Rosenau. 1973. Four factor response surface experimental design for evaluating the role of processing variables upon protein denaturation in heated whey systems. J. Dairy Sci. 56:76.

75 Nuys, V. 1980. Lactose intolerance. Nutr. and the M.D. 6(1):3.

76 Okos, M. R., E. A. Grulke, and A. Syverson. 1978. Hydrolysis of lactose in acid whey using β-galactosidase adsorbed to a phenol formaldehyde resin. J. Food Sci. 43:566.

77 O'Leary, V. S., and J. H. Woychik. 1976. Utilization of lactose, glucose, and galactose by a mixed culture of Streptococcus thermophilus and Lactobacillus bulgaricus in milk treated with lactase enzyme. Applied and Environmental Micro. 32(1):89.

78 O'Leary, V. S., and J. H. Woychik. 1976. A comparison of some chemical properties of yogurt made from control and lactase-treated milks. J. Food Sci. 41:791.

79 Pazur, J. H. 1954. The mechanism of enzymatic synthesis of galactosyl oligosaccharides. J. Biol. Chem. 208:439.

80 Pitcher, W. H., Jr. 1975. Hydrolysis of whey by immobilized lactase. Am. Dairy Rev. 37(9):34 B.

81 Pomeranz, Y. 1964. Lactase (β-D-galactosidase):1. Occurrence and properties. Food Technol. 18(5):88.

82 Pomeranz, Y. 1964. Lactase (β-D-galactosidase):11. Possibilities in the food industries. Food Technol. 96:690.

83 Prodanski, P. G. 1970. Utilization of proteins from whey, buttermilk, and skim milk. Moloch. Prom. 30(9):44 (Dairy Sci. Abstr. 32:25).

84 Reddy, V. 1974. Milk intolerance and lactose intolerance. Cajanus VII(2):50.

85 Reddy, V., and J. Pershad. 1972. Lactase deficiency in Indians. Am. J. Clin. Nutr. 25:114.

86 Roberts, H. R., and J. D. Pettinati. 1957. Concentration effects in the enzymatic conversion of lactose to oligosaccharides. J. Agr. Food Chem. 5:130.

87 Rosenweig, N. S. 1969. Adult human milk intolerance and intestinal lactase deficiency: a review. J. Dairy Sci. 52:585.

88 Sandine, W. E., C. Daly, P. R. Elliker, and E. R. Vedamuthu. 1972. Causes and control of culture - related flavor defects in cultured dairy products. J. Dairy Sci. 55:1031.

89 Schingoethe, D. J. 1976. Whey utilization in animal feeding: a summary and evaluation. J. Dairy Sci. 59:556.

90 Shah, N. O., and T. A. Nickerson. 1978. Functional properties of hydrolyzed lactose: Relative sweetness. J. Food Sci. 43(5):1575.

91 Shahani, K. M., and R. C. Chandan. 1979. Nutritional and healthful aspects of cultured and culture containing dairy foods. J. Dairy Sci. 62:1685.

92 Shahani, K. M., B. N. Mathur, and A. Kilara. 1978. Utilization of whey as a human food. Cult. Dairy Prod. J. 13(2):7.

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

94 Singh, J., A. Khanna, and H. Chander. 1980. Effect of incubation temperature and heat treatments of milk from cow and buffalo on acid and flavor production by Streptococcus thermophilus and Lactobacillus bulgaricus. J. Food Prot. 43:399.

95 Singh, J., and D. K. Sharma. 1982. Yogurt starters in skim milks. 1. Acid and flavor production and proteolytic activity by yogurt starters. Cult. Dairy Prod. J. 17(1):22.

96 Speck, M. L. 1975. Interactions among Lactobacilli and man. J. Dairy Sci. 59:338.

97 Speck, M. L. 1979. Yogurt qualities affected by starters and processing. Dairy Ind. International 44(3):5.

98 Speck, M. L., and J. W. Geoffrion. 1980. Lactase and starter culture survival in heated and frozen yogurts. J. Food Prot. 43(1):26.

99 Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics. 2nd ed., McGraw-Hill Book Company, Inc., New York, NY.

100 Steinitz, W. S. 1977. Why use stabilizers in cultured products, their dressings and fruits? Cult. Dairy Prod. J. 12(4):22.

101 Tamime, A. Y. 1977. The behavior of different starter cultures during the manufacture of yogurt from hydrolyzed milk. Dairy Ind. International 42(8):7.

102 Tamime, A. Y. 1978. The production of yogurt and concentrated yogurt from hydrolyzed milk. Cult. Dairy Prod. J. 13(3):16.

103 Tamime, A. Y., and H. C. Deeth. 1980. Yogurt: Technology and biochemistry. J. Food Prot. 43:939.

104 Tamime, A. Y., and R. K. Robinson. 1978. Some aspects of the production of a concentrated yogurt (labneh) popular in the Middle East. Milchwissenschaft 33(4):209 (English version).

105 Thompson, M. P., and D. P. Brower. 1976. Hydrolyzed lactose cultured dairy products. 1. Manufacture of cheddar cheese. Cult. Dairy Prod. J. 11(1):22.

106 Thompson, M. P., and D. M. Gyuricsek. 1974. 1. Manufacture of yogurt, buttermilk, and cottage cheese from hydrolyzed lactose milks. J. Dairy Sci. 57:584.

107 Toba, T., and S. Adachi. 1978. Hydrolysis of lactose by microbial β-galactosidases. Formation of oligosaccharides with special reference to 2-O-β-D-galactopyranosyl-D-glucose. J. Dairy Sci. 61:33.

108 Todoric, R., and K. Savadinovic. 1973. Use of dried whey in yogurt manufacture and its effect on acidity and consistency. Mljekarstvo. 23(4):78 (Dairy Sci. Abstr. 35:449).

109 Vakaleris, D. G., and W. V. Price. 1959. A rapid spectrophotometric method for measuring cheese ripening. J. Dairy Sci. 42:264.

110 Vedamuthu, E. R. 1974. Cultures for buttermilk, sour cream, and yogurt with special comments on acidophilus yogurt. Cult. Dairy Prod. J. 9(1):16.

111 Vedamuthu, E. R. 1978. Natural (unhydrolyzed) milk versus lactose-hydrolyzed milk for cultured dairy products: physiological and practical implications for the starter industry. J. Food Prot. 41:654.

112 Vujicic, I. F., A. Y. Lin, and T. A. Nickerson. 1977. Changes during acid hydrolysis of lactose. J. Dairy Sci. 60:29.

113 Webb, B. H. 1972. Recycling whey for profitable uses. Am. Dairy Rev. 34(6):32 A.

114 Webb, B. H., and E. O. Whittier. 1948. The utilization of whey: a review. J. Dairy Sci. 31:139.

115 Weetal, H. H., N. B. Havewala, W. H. Pitcher, C. C. Detar, W. P. Vann, and S. Yaverbaum. 1974. Preparation of immobilized lactase and its use in the enzymatic hydrolysis of acid whey. Biotech. & Bioeng. 16:295.

116 Weiner, G. D. 1977. Changing times for the whey industry. Am. Dairy Rev. 39(12):42 B.

117 Weisberg, S. M., and H. I. Goldsmith. 1969. Whey for foods and feeds. Food Technol. 23:186.

118 Wendorff, W. L., C. H. Amundson, and N. F. Olson. 1970. The effect of heat treatment of milk upon the hydrolyzability of lactose by the enzyme lactase. J. Milk Food Technol. 33:377.

119 Wendorff, W. L., C. H. Amundson, N. F. Olson, and J. C. Garver. 1971. Use of yeast β-galactosidase in milk and milk products. J. Milk Food Technol. 34:294.

120 Whalen, C. A. 1982. Whey-caseinate blends and lactose hydrolysis in yogurt manufacture. M. S. thesis. Dairy Science Dept., South Dakota State University, Brookings.

121 Whelan, E., and F. J. Stare. 1980. Lactose intolerance: who has it and why? Weight Watchers 13(10):50.

122 Wierzbicki, L. E., and F. V. Kosikowski. 1973. Lactase potential of various microorganisms grown in whey. J. Dairy Sci. 56:26.

123 Wierzbicki, L. E., and F. V. Kosikowski. 1973. Formation of oligosaccharides during β-galactosidase action on lactose. J. Dariy Sci. 56:1400.

124 Woychik, J. H., and M. V. Wondolowski. 1973. Lactose hydrolysis in milk and milk products by bound fungal β-galactosidase. J. Milk Food Technol. 36(1):31.

APPENDIX

APPENDIX FIGURE 1. American Dairy Science Association Product Judging Card for Swiss style yogurt.

FLAVOR _____ CONTEST CONTESTANT NO. _____
DATE _____ ADSA SWISS STYLE YOGURT SCORE CARD DISA _____

PERFECT SCORE	CRITICISMS	SAMPLE NO. 1	2	3	4	5	6	7	8	9	10	TOTAL GRADES
FLAVOR 10	CONTESTANT SCORE →											
	GRADE SCORE											
	GRADE CRITICISM											
	BITTER											
	COOKED											
NO CRITICISM 10	COARSE											
	FOREIGN											
	HIGH ACID											
	LACKS FINE FLAVOR											
	LACKS FLAVORING											
	LACKS FRESHNESS											
NORMAL RANGE	LACKS SWEETNESS											
1-10	LOW ACID											
	OLD INGREDIENT											
	OXIDIZED											
	RANCID											
	TOO HIGH FLAVORING											
	TOO SWEET											
	UNNATURAL FLAVORING											
	UNCLEAN											
BODY AND TEXTURE 5	CONTESTANT SCORE →											
	GRADE SCORE											
	GRADE CRITICISM											
NO CRITICISM 5	GEL-LIKE											
	GRAINY											
	LUMPY											
NORMAL RANGE	ROPY											
1-5	TOO FIRM											
	WEAK											
APPEARANCE 5	CONTESTANT SCORE →											
	GRADE SCORE											
	GRADE CRITICISM											
NO CRITICISM 5	ATYPICAL COLOR											
	COLOR LEACHING											
	EXCESS FRUIT											
NORMAL RANGE	FREE WHEY											
	LACKS FRUIT											
	SHRUNKEN											
	SURFACE GROWTH											
TOTAL 100	TOTAL SCORE OF EACH SAMPLE →											
	TOTAL GRADE PER SAMPLE											

CODE	GRADE
1	
2	
3	
TOTAL	
RANK	

TEAM RANK

FINAL GRADE _____

RANK _____

APPENDIX TABLE 1. Component formulas for whey and NDM hydrolyzed lactose bases in 1 kg (2.2 lb) batch.

Ingredients	No hydrolysis	50% hydrolysis	75% hydrolysis
	gram		
12:0			
NDM	120	130	140
Dry whey	0	0	0
Anhydrous milk fat	20	20	20
Sucrose	40	30	20
Stabilizer	5	5	5
Distilled water	815	815	815
TOTAL	1000	1000	1000
9:3			
NDM	90	100	110
Dry whey	30	30	30
Anhydrous milk fat	20	20	20
Sucrose	40	30	20
Stabilizer	5	5	5
Distilled water	815	815	815
TOTAL	1000	1000	1000
6:6			
NDM	60	70	80
Dry whey	60	60	60
Anhydrous milk fat	20	20	20
Sucrose	40	30	20
Stabilizer	5	5	5
Distilled water	815	815	815
TOTAL	1000	1000	1000

APPENDIX TABLE 2. Analysis of variance[a] of total solids in yogurts.

Source	DF	SS	F
Replication	4	.2190	....
Whey content	2	.0160	.58[NS]
Replication x whey level	8	.1106	....
Hydrolysis of lactose	2	.0214	1.07[NS]
Replications x hydrolysis of lactose	8	.0801	....
Whey content x hydrolysis of lactose	4	.0529	1.17[NS]
Replication x whey content x hydrolysis of lactose	16	.1816	....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

[NS]Not significant.

APPENDIX TABLE 3. Analysis of variance[a] of lactose percentages in three yogurt formulations with 0, 50, or 75% of lactose hydrolyzed.

Source	DF	SS	F
Replication	4	.9847	.....
Whey content	2	16.5677	504.17**
Replication x whey content	8	.2055	.....
Hydrolysis of lactose	2	5.5582	329.34**
Replication x hydrolysis of lactose	4	.0298	.....
Whey level x hydrolysis of lactose	4	.2382	17.91**
Replication x whey content x hydrolysis of lactose	8	.0192	.....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

**Highly significant (P<.01).

APPENDIX TABLE 4. Analysis of variance[a] of average actual percentages of lactose hydrolysis in yogurts manufactured by three formulations in which 0, 50, or 75% of the lactose was to be hydrolyzed.

Source	DF	SS	F
Replication	4	31.8000	....
Whey content	2	4.2667	1.19[NS]
Replication x whey content	8	14.4000	....
Hydrolysis of lactose	1	5306.7000	339.81[**]
Replication x hydrolysis of lactose	4	62.4667	....
Whey content x hydrolysis of lactose	2	5.6000	.87[NS]
Replication x whey content x hydrolysis of lactose	8	25.7333	....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

[NS]Not significant.

[**]Highly significant (P<.01).

APPENDIX TABLE 5. Analysis of variance[a] of average protein percentages in yogurt bases.

Source	DF	SS	F
Replication	4	.2001	.....
Whey content	2	10.5972	787.44**
Replication x whey content	8	.0538	.....
Hydrolysis of lactose	2	4.5195	898.30**
Replication x hydrolysis of lactose	8	.0201	.....
Whey content x hydrolysis of lactose	4	.0093	1.06NS
Replication x whey content x hydrolysis of lactose	16	.0348	.....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

**Highly significant (P<.01).

NSNot significant.

APPENDIX TABLE 6. Analysis of variance[a] of titrable acidities of yogurts.

Source	DF	SS	F
Replication	4	.0671	.....
Whey content	2	.1869	49.72**
Replication x whey content	8	.0150	.....
Hydrolysis of lactose	2	.1756	23.94**
Replication x hydrolysis of lactose	8	.0293	.....
Whey content x hydrolysis of lactose	4	.0011	.17NS
Replication x whey content x hydrolysis of lactose	16	.0270	.....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

**Highly significant (P<.01).

NSNot significant.

APPENDIX TABLE 7. Analysis of variance[a] of pH values of yogurts.

Source	DF	SS	F
Replication	4	.0230	.....
Whey content	2	.0918	73.39**
Replication x whey content	8	.0050	.....
Hydrolysis of lactose	2	.0846	28.35**
Replication x hydrolysis of lactose	8	.0119	.....
Whey content x hydrolysis of lactose	4	.0016	1.08NS
Replication x whey content x hydrolysis of lactose	16	.0059	.....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

**Highly significant (P<.01).

NSNot significant.

APPENDIX TABLE 8. Analysis of variance[a] of water soluble nitrogen contents of uncultured yogurt mixes.

Source	DF	SS	F
Replication	4	.0017	.....
Whey content	2	.0154	27.26**
Replication x whey content	8	.0023	.....
Hydrolysis of lactose	2	.0085	46.55**
Replication x hydrolysis of lactose	8	.0007	.....
Whey content x hydrolysis of lactose	4	.0003	.64NS
Replication x whey content x hydrolysis of lactose	16	.0016	.....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

**Highly significant (P<.01).

NSNot significant.

APPENDIX TABLE 9. Analysis of variance[a] of water-soluble nitrogen values in cultured yogurts.

Source	DF	SS	F
Replication	4	.0159	.....
Whey content	2	.0088	18.07**
Replication x whey content	8	.0019	.....
Hydrolysis of lactose	2	.0066	9.97**
Replication x hydrolysis of lactose	8	.0027	.....
Whey content x hydrolysis of lactose	4	.0002	.96NS
Replication x whey content x hydrolysis of lactose	16	.0008	.....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

**Highly significant ($P<.01$).

NSNot significant.

APPENDIX TABLE 10. Analysis of variance[a] of flavor scores of fresh yogurts.

Source	DF	SS	F
Replication	4	.2371	....
Whey content	2	1.7372	6.11*
Replication x whey content	8	1.1373	....
Hydrolysis of lactose	2	.4618	.57NS
Replication x hydrolysis of lactose	8	3.2365	....
Whey content x hydrolysis of lactose	4	3.2549	4.79**
Replication x whey content x hydrolysis of lactose	16	2.7168	....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

*Significant (P<.05).

**Highly significant (P<.01).

NSNot significant.

APPENDIX TABLE 11. Analysis of variance[a] of flavor scores of yogurts after 1 wk storage.

Source	DF	SS	F
Replication	4	29.6044	....
Whey content	2	3.8317	7.89*
Replication x whey content	8	1.9426	....
Hydrolysis of lactose	2	8.3779	5.63*
Replication x hydrolysis of lactose	8	5.9571	....
Whey content x hydrolysis of lactose	4	4.1091	2.73NS
Replication x whey content x hydrolysis of lactose	16	6.0239	....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

*Significant ($P<.05$).

NSNot significant.

APPENDIX TABLE 12. Analysis of variance[a] of average body and texture scores of fresh yogurt.

Source	DF	SS	F
Replication	4	6.9784	....
Whey content	2	2.3405	1.83[NS]
Replication x whey content	8	5.1259	....
Hydrolysis of lactose	2	1.4585	5.30[*]
Replication x hydrolysis of lactose	8	1.1011	....
Whey content x hydrolysis of lactose	4	1.9861	4.21[*]
Replication x whey content x hydrolysis of lactose	16	1.8879	....

[a]Analysis of variance using 3 x 3 factorial design with five replicates.

[*]Significant ($P<.05$).

[NS]Not significant.

APPENDIX TABLE 13. Analysis of variance[a] of viscosities of yogurts.

Source	DF	SS	F
Replication	1	41102222	.....
Whey content	2	2514773333	121.11**
Replication x whey content	2	20764444	.....
Hydrolysis of lactose	2	1869653333	27.27*
Replication x hydrolysis of lactose	2	68551111	.....
Whey content x hydrolysis of lactose	4	23893333	1.24NS
Replication x whey content x hydrolysis of lactose	4	19342222	.....

[a]Analysis of variance using 3 x 3 factorial design with two replicates.

*Significant (P<.05).

**Highly significant (P<.01).

NSNot significant.