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# Whey-Caseinate Blends and Lactose Hydrolysis in Yogurt Manufacture

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# WHEY-CASEINATE BLENDS AND LACTOSE HYDROLYSIS IN YOGURT MANUFACTURE

BY

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WHEY-CASEINATE BLENDS AND LACTOSE
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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 a highly nutritious cultured dairy product which has been consumed for centuries, particularly in Eastern Europe. Although per capita consumption in the United States is still far below that of most European countries, yogurt sales in the U.S.A. have increased phenomenally during the past three decades. Yogurt sales in the U.S.A. totaled 7.7 million kg in 1955; 31.8 million kg in 1966; and approximately 227 million kg by 1976 (41). Per capita sales increased 211% from 1970 to 1980 (45).

Yogurt is generally manufactured from milk or lowfat milk which has been fortified with extra milk solids. Nonfat dry milk (NDM) is the usual source of these milk solids, but NDM has been steadily increasing in price. The price of NDM has increased from $1.76/kg in 1979 (14) to $2.11/kg in 1982 (15), an increase of 20%. Less expensive but compositionally and nutritionally equivalent substitutes for NDM in the manufacture of yogurt, such as certain modified whey products, would seem to present an economically attractive alternative to the yogurt processor.

The Whey Products Institute estimates approximately 18.2 million kg of whey were produced in the U.S.A. in 1980, of which less than half was processed and used in human foods (4). About 55% of the processed whey was concentrated and spray dried into a variety of products. Greater usage of these products in human foodstuffs has become possible because of greatly improved product quality attributed to better sanitation, handling, and processing methods. Many

nutritious whey products, such as partially delactosed or demineralized whey powders and whey protein concentrates (WPC) with the proteins in an undenatured form, are available to the dairy processor. Depending upon the processing methods, the whey protein products possess a wide range of functional and nutritional properties which make possible a variety of applications in food products (16).

A major component of the milk solids in yogurt is the disaccharide, lactose. Lactase enzyme hydrolyzes lactose into glucose and galactose which individually and together are sweeter than lactose itself. The resulting sugar mixture is also more soluble (31), easily digestible by lactose intolerant individuals, and is more readily fermented by lactic acid organisms (52). In general, northern Europeans and their descendants and members of two African tribes are the only persons who retain their childhood ability to digest lactose as adults. An estimated 30 million Americans can not digest lactose properly and among certain ethnic groups (Blacks, Asians, Mediterraneans, Jews, Southern and Central Europeans, and American Indians), 70% have difficulty digesting lactose as adults (9). Hydrolysis of at least part of the lactose in milk prior to its manufacture into various products not only may be a potential partial solution to the lactose intolerance problem (31), but it may result in improved products with increased sweetness without increased calories (17, 31), increased carbohydrate solubility, and better mouth feel and body (3, 31).

One objective of this research was to determine the feasibility of using two reconstructed milk products (RMP) as economical

replacements for NDM in fortifying 2% lowfat milk for yogurt. These RMP's were spray dried blends of whey proteins and caseinates. A second objective was to determine if concomitantly, partial enzymatic hydrolysis of lactose would afford the same degree of sweetness in yogurts containing less sucrose than nonhydrolyzed yogurts. A yogurt with less sucrose should be attractive to consumers seeking products with fewer calories and one with lowered lactose levels would be more desirable to those deficient in lactase. Yogurts were manufactured with several concentrations of these variable factors and analyzed for composition. The yogurts were evaluated for flavor by a panel of dairy science faculty and by randomly selected volunteers in a consumer panel.

# LITERATURE REVIEW

Yogurt is an ancient cultured dairy product and is still a traditional food and beverage in the Balkans and the Middle East. Yogurt plays an important role in the diets of many Europeans, especially Bulgarians; and it is becoming increasingly important in the diets of individuals in many other countries (60). Although yogurt has been considered for many years to be a nutritious, refreshing, and highly desirable food, Americans "discovered" yogurt only recently. Yogurt sales have recently shown the largest gains of any product in the refrigerated dairy case (11). Apparently the dramatic increases in yogurt sales can be partly attributed to the fairly recent addition of flavorings, fruit, and sugar to yogurt, making it more palatable and pleasing to consumers (28).

The yogurt boom in America began in the late 1960's (35) and while per capita consumption is still far below that of most European countries, sales of yogurt have increased more than the sale of any other dairy product. The growth of sales of yogurt in the United States can only be reported as phenomenal; sales increased 270% from 1962 to 1970, then increased 260% from 1970 to 1977 (33). Sales totaled 267.4 million kg in 1980 (45). Per capita sales of yogurt in the U.S. increased from .05 kg in 1955 (57) to 1.03 kg in 1976, and by 1980 it had increased to 1.2 kg (45). In 1978, the average per capita consumption of yogurt in the Netherlands, France, United Kingdom, Switzerland, Belgium, Austria, Denmark, and Italy was 5.2 kg (39).

The Manufacture of Yogurt

Manufacturing good quality yogurt requires quality ingredients and exact attention to details regardless of the style of yogurt made (68). Whole or lowfat milk may be used as the basic ingredient to which nonfat milk solids, stabilizers, and sugar usually will be added. The mix is then pasteurized, homogenized, cooled to incubation temperature, and culture added. Incubation for development of acid and flavor may be done either before or after the yogurt is dispensed into its final packages (68).

Most manufacturers use whole or partially skimmed milk for yogurt. Nearly 85% of the fresh yogurt is made from lowfat milk and only 14% is made from whole milk (33). The presence of milk fat in yogurt directly affects the mouthfeel; the texture will be smoother with a higher percentage of fat in the mix. Milk fat contents between 2 and 4% fat are reported to be optimum (10). The majority of American-made yogurts contain 1.0 to 2.0% fat but even these amounts have a beneficial effect on the body and texture (50). It is of primary importance that the raw milk be of high quality and be collected, stored, and handled under favorable conditions, because off-flavors in the raw milk can be carried into the finished yogurt (10).

The body characteristics of yogurt are influenced directly by the milk solids of the milk and by the stabilizer system (10). The consistency and aroma of yogurt are also affected by milk solids and can be enhanced by an increase in the level of total solids (60). Full cream, skim, or buttermilk powder is generally used to fortify or

increase the total solids to produce thick, smooth yogurt (60). The recommended level of addition of nonfat milk solids is around 3 to 4% (7, 58) with a range from as little as 1% (26) to as high as 6% (60). Enough milk solids must be added to increase the total milk solids in the mix into the range of 12 to 15% (10, 11, 40). Milk solids above 15% will yield a firm, heavy body while solids around 9% will yield a weak bodied yogurt (10). Vacuum concentration or ultrafiltration may also be used to increase the total solids for the production of thick, smooth yogurt (60).

Stabilizers. When properly employed, stabilizers are useful in improving the body, texture, mouthfeel, and appearance of yogurts (35). Although stabilizers are widely used, a firm bodied product can be made without the use of stabilizers. Gelatin, starch, vegetable gums, and pectin are widely used as yogurt stabilizers (10). Gelatin has been found to afford the best improvement in yogurt texture (11, 54). The use of agar and pectin produced satisfactory body and texture but delayed acid production. Alginates, carboxymethylcellulose, locust bean, carrageenan, and guar gum caused wheying off and retarded acid production (54). The recommended levels of gelatin usage are .3 to .8%. The quantity of stabilizer used depends upon the type of yogurt produced and upon the choice of stabilizer (35).

Sugar Content. Cane, beet, or corn sugars may be added to subdue the sharp acid flavor or to improve the flavor of fruit-flavored

yogurts. Enough sugar should be added to mask the full degree of acidity while retaining the characteristic yogurt flavor with a desirable acid-sugar blend. A range of 4 to 6% sugar is recommended when the final pH is expected to be 4.0 to 4.2 (40). Culture growth may be inhibited by a sugar percentage of nine or above (11).

Heat Treatment. Heat treatment of the yogurt mix is considered quite critical and is best accomplished at temperatures between 82 to 92°C for 30 min (10, 11, 35, 40). The primary purpose of pasteurization is destruction of microorganisms which cause spoilage in the finished product or which may be pathogenic to man. Almost all organisms except vegetative spore formers are killed during pasteurization (60). Pasteurization also denatures the whey proteins, which markedly increases their water binding capacity (40) and improves the gel structure of the yogurt (68). Pasteurization promotes proper hydration of the stabilizer, liberates free amino acids from the milk protein which facilitate the growth of Lactobacillus bulgaricus, and fosters less wheying off or syneresis (11). Use of pasteurization temperatures above 90°C for 30 min increases the risk of syneresis caused by excessive denaturation of the whey proteins. This excessive denaturation results in reduction of the water binding capacity of the proteins. Syneresis develops with a loss of water binding capacity and the gel structure of the yogurt becomes weak and fragile (10).

Culturing. Following heat treatment, the yogurt mix is cooled to a temperature compatible with the culture inoculation method and incubation condition of choice. The inoculation temperature is generally 3.3°C above the incubation temperature if the yogurt is incubated in its final package. However, the mix is to be cooled directly to and held at the desired incubation temperature if incubation is to be completed in vats prior to dispensing the yogurt into packages (10).

The yogurt mix is inoculated with a 1:1 ratio of Streptococcus thermophilus and Lactobacillus bulgaricus organisms. The ratio of cocci to rods is important and should not be above 3:2 in the final product for optimum results. An imbalance of S. thermophilus to L. bulgaricus may result in a coarse flavor from the over production of aroma compounds by L. bulgaricus. Over production of acetaldehyde is especially critical since it is the characteristic aroma compound in yogurt (40).

Either of two incubation temperature ranges may be used: 41 to 42°C or 30 to 32°C. Temperatures above 42°C will allow the Lactobacillus culture to grow to excessive numbers with the production of inordinate amounts of lactic acid which causes a sharper acid flavor, and contributes to syneresis and poor gel development (10). The lower range of incubation favors a reduced rate of lactic acid production and the formation of a firmer, more desirable body (10, 40). Incubation temperatures of 41 to 42°C tend to favor the two cultures equally and yield the desired 1:1 ratio. As noted above, a range of 42 to 46°C will favor the Lactobacillus culture; while below 41°C the

Streptococcus culture will be favored and dominate the yogurt (10). Lack of flavor, bitterness, or too high acid level can result should either species be allowed to dominate (11). The higher incubation temperatures require a starter inoculum rate of 1 to 5% while the lower incubation temperatures require inoculations of only .01 to .25% (50).

Changes During Incubation. Once the cultures are inoculated into the yogurt base, a symbiosis occurs between S. thermophilus and L. bulgaricus. Pasteurization or heat treatment serves to stimulate growth of the starter cultures through destruction of heat-labile inhibitors, partial protein hydrolysis, and expulsion of oxygen (64). The Lactobacilli hydrolyze the casein and release certain amino acids, particularly valine, which stimulate the Streptococci by serving as essential growth requirements (11). S. thermophilus has a short lag phase and outgrows L. bulgaricus until the ratio of cocci to rods is about 3:1 at a pH of about 5.0 to 5.5. The formation of lactic acid from lactose lowers the pH which inhibits further growth of S. thermophilus (1) and also produces anaerobic conditions along with compounds similar to formic acid (60). These conditions allow L. bulgaricus to proliferate and produce more lactic acid (1), lowering the pH from 5.0 to about 3.95 to 4.4 while producing acetaldehyde (10). The ratio of cocci to rods will be approximately 1:1 when the lactic acid finally inhibits further growth of the Lactobacilli (1).

The starter culture has two major roles during the manufacture

of yogurt; one is to produce lactic acid and the other is to develop the flavor of the product. Carbonyl compounds such as acetaldehyde, acetone, acetoin, and diacetyl are the major flavor compounds in yogurt and are formed by fermentation of the lactose by the Lactobacilli. The most important chemical process occurring during yogurt manufacture is the production of lactic acid which contributes the sharp, acid taste to yogurt and also contributes to the typical aromatic flavor. The casein micelle is destabilized by lactic acid which leads to coagulation of the casein protein and formation of the yogurt gel (60).

Once a titratable acidity of .85 to .90% lactic acid is reached, incubation is terminated and the yogurt must be cooled rapidly to below 21°C to stop acid development (11) and to begin conditioning the proteins for better whey retention (10). At this point, yogurt manufactured by the vat method would be carefully dispensed into its final package. The product is then cooled as quickly as possible to its storage temperature of 4 to 5°C. Depending upon consumer preference, the final yogurt may have an acidity between .90 and 1.25% (35) and the pH may range from 3.9 to 4.2 (68). A weak coagulum results from a pH above 4.5 (35).

Whey Supplies and Utilization

Whey is the yellowish-green solution remaining after the removal of the milk fat and the casein from milk during cheesemaking or the manufacture of casein and related products. Cheese production in

the U.S.A. is increasing; per capita sales increased 53% from 1970 to 1980 (45). With more cheese being produced annually, it follows that more whey is produced since about 9 kg of whey are generated for every kg of cheese manufactured. For example, 1.8 billion kg of all types of cheese were produced in 1976 generating 15.4 billion kg of whey (12). By 1980, those figures had risen to approximately 2.3 billion kg of cheese and about 19 billion kg of whey (45).

The disposal of whey has been a problem since the beginning of cheesemaking. Over the years, fluid whey has traditionally been fed to farm animals, spread over fields, or dumped into the nearest waterway. Stricter water pollution laws, higher disposal costs, and recognition of the amounts of valuable food nutrients contained in fluid whey have helped increase the utilization of whey for human foods or animal feeds (34). Today only about 56% of all whey is utilized in human nutrition or in animal feed and the remainder is disposed of in some manner (47) wasting many tons of valuable food nutrients.

## The Composition and Nutritional Value of Whey

Average liquid whey contains about 6.5% total solids which represent about 55% of the original milk nutrients. About 80% of the whey produced is sweet whey of which the major components are lactose (4.85%), protein (.8%), minerals (.5%), and fat (.5%) (34). These nutrients can supply important needs in the human diet, including minerals such as calcium, phosphorus, magnesium, potassium, and sodium; and vitamins such as riboflavin, pantothenic acid, thiamine, and

niacin (13). The whey proteins, α-lactalbumin and β-lactoglobulin, are of excellent nutritional quality and have been shown to be highly superior to most other proteins in meeting human nutritional needs (22, 23, 38, 66). In one study (38), laboratory rats were fed diets containing either 12% casein protein or 12% whey protein. While weight gains during the 1st wk were similar with both proteins, the weight gains with whey protein were significantly greater than with casein during the 2nd wk. Similar results were obtained with diets of 10% whey or 10% casein protein. The authors concluded that the effects of some limiting amino acids become apparent only at later stages.

In a different study (66), weanling rats were fed diets containing either 10% casein protein or 10% whey protein concentrate (WPC). Results showed that between the 4th and 15th wk, the growth rate of the rats on the WPC diet exceeded that of the rats on the casein diet by 24%. The protein efficiency ratio (PER) for soluble lactalbumin was determined to exceed the PER of casein by 24%. Phosphorus and calcium availability studies (65) showed the protein and minerals of soluble WPC to be completely available in animal and human nutrition.

The high lysine content of whey protein is one of its most valuable nutritional features. Forsum and Hambraeus (23) found a high available lysine content in several whey products, including some WPC's. From their results, they suggested the nutritional value of the whey protein is retained even after considerable heat treatment.

Whey protein contains adequate amounts of the amino acids essential for human nutrition; whereas different vegetable proteins lack one or more essential amino acids, which impedes protein utilization in vivo (65). For example, up to 60% of the potential nutritive value from cereal proteins is not utilized in the absence of proper supplementation because cereal grains lack several essential amino acids. On the other hand, the lactalbumin fraction possesses large surpluses of five essential amino acids and consequently may be the perfect supplement for many proteins in the world's food supply. The minimum adult daily requirement of the essential amino acids can be supplied by 14.5 g of α-lactalbumin (65).

Food Uses of Whey Products

The utilization of whey is steadily increasing as technology is either developed or refined to utilize whey for human food. Greater utilization is also attributed to increased awareness of the unique biological, nutritional, and functional characteristics of whey components (42), plus the fact that whey solids are the cheapest dairy ingredient which can be used by the food processor (13). Valuable whey solids may be recovered by concentration, drying, lactose crystallization, demineralization, protein precipitation, reverse osmosis/ultrafiltration, or by gel filtration. All of the component whey solids are recovered by concentration, drying, and reverse osmosis; while the remaining techniques are fractionating systems used for recovering part of or specific whey components (43).

Most food processors use whey products as replacements for NDM; however, they can be used advantageously in many food formulations with favorable functional benefits. Since there is no casein in whey powder to mask flavors, whey accentuates the flavor of fruits and spices (43). Whey solids also help retain moisture and freshness while adding a natural tenderness or shortness to many products. Additionally, all baked items containing whey demonstrate faster browning and an even color development which are attributed largely to the lactose and whey proteins (13).

Whey powder, when incorporated into cooked foods such as gravies and sauces, reduces the tendency of sticking to the pan since the whey proteins do not adhere and char on the pan as readily as do other proteins. Spray dried whey can replace NDM in food products, affording the previously mentioned benefits while maintaining sound nutritional properties in various types of foods such as ice cream, dry mixes, baked goods, cooked foods, confections, and frozen foods (13). The largest users of edible whey in the U.S.A. are bakeries which can exploit its favorable qualities and minimize the effects of its deficiencies by combinations with other ingredients that can compensate for these deficiencies (43). Increased usage of whey solids in human foods is expected to continue, especially if NDM prices continue to rise and pollution prevention continues to be emphasized. New uses will undoubtedly be developed for whey solids which are available in a wide variety of product forms (43).

Whey Protein Concentrates

The production of fractionated whey concentrates has risen greatly recently as engineering and technical problems were solved (33) in addition to a better understanding of the nutritional, biological, and functional characteristics of whey components (42). Since various vegetable proteins lack one or more essential amino acid, much attention has been focused on whey proteins which have adequate levels of essential amino acids, are easily digestible, and are highly nutritional and physiologically complete (42).

The whey proteins have been commercially available for many years but in a heat-denatured form. These heat-denatured proteins were only available as a brownish, gritty, and completely insoluble powder. Thus, this nutritionally superior protein found limited use in food manufacturing (66).

Over the past 20 years, considerable effort has been made to invent and refine processes for recovering whey proteins in a native and functional state (47). Methods for large-scale production of undenatured WPC's have recently been developed. Much activity has been devoted to the production of these WPC's which are powders containing high concentrations of whey proteins, generally from 35 to 80% on a dry weight basis (16). The composition of WPC's is mostly dependent upon the preparatory process. The protein concentration may be increased by altering the fractionation process to remove more of the lactose and milk salts. Whey protein concentrates with greater than 50 to 60% protein probably will not be produced in any great amount

because of the high cost of production (47) which will rise sharply as the concentration of protein is increased.

There are a number of methods that can be used to prepare WPC's: ultrafiltration, electrodialyses, filtration, ion complexing, metaphosphate precipitation, carboxymethyl cellulose complexing, alcohol precipitation, and ion exchange. The greatest interest today is in the ultrafiltration-derived products (16). Care must be exercised to minimize protein denaturation by heat and/or mechanical trauma.

Functional Properties of WPC's

In contrast to the insolubility and gritty character of denatured whey proteins, the undenatured form has many desirable physical properties (65). Whey protein concentrates are not only relatively bland in flavor, but if prepared with minimal heat treatment and under careful processing conditions, they are relatively free of off-flavors and odors. In addition to maximum nutritional benefits, undenatured whey proteins have many functional advantages in food systems. The major functional properties possessed by whey proteins are solubility, stabilization, emulsification, foam expansion, water sorption, and gelation (47).

Whey proteins can be completely soluble and exist in a non-aggregated state for optimal functionality in foams, emulsions, and beverages if they are undenatured. Because of their solubility they can mix completely with other ingredients of food formulations

throughout the pH range of three to eight. If the whey proteins are denatured, they aggregate and precipitate and fail to provide adequate functionality (47).

Proteins are able to function as emulsifying agents but the size, shape, and solubility of the protein molecule, plus pH, temperature and ionic background effects have considerable influence in their actual emulsifying capacity. Whey proteins prepared by different processes have variable emulsifying capacities depending upon previous processing treatments. Treatments that promote protein denaturation and aggregation will have a detrimental effect on emulsifying capacities (48). Whey protein concentrates generally have good emulsification properties over a wide pH range, whereas milk and soya proteins are very pH dependent. Whey protein concentrates have a lower emulsion capacity than does casein (47).

Whey proteins are usually good foaming agents but are not as suitable for this application as are caseinates, egg white proteins, and hydrolyzed soy proteins (47), but whey protein foams are unstable when subjected to heat such as in cake baking (43). However, WPC's can be used in bread baking and other raised baked products if the proteins have been previously heat denatured. An unstable foam structure that would have depressed loaf volume during baking would then be unable to form. The foaming properties of WPC's are affected by a number of compositional and processing conditions such as pH, redox potential, calcium, heat denaturation, enzymic hydrolysis, and

residual lipids. Foaming properties are also influenced by the concentration of proteins, sugars, and other ingredients of the food formulations (47). Heat treatments and pH adjustments of whey proteins are reported to be effective in producing good foams. Limited heat treatment apparently causes partial denaturation which increases water affinity and influences permanency of foams. Temperatures of 65 to 70°C greatly improved foaming properties while higher temperatures impaired foaming properties (44).

Heat denatured whey proteins have the ability to hold water to essentially the same degree as undenatured whey proteins. Apparently entrapment of water is accomplished through a network of cellular protein filaments into a classical gel structure rather than a true binding of unfreezable water (44). The affinity of whey protein for water is measured by the strength of the gels formed by heating WPC or egg white added to skim milk since the formulation of most gels involve heat treatment. In one experiment, various amounts of WPC were added to skim milk to total .5 to 2% whey protein based on the total volume of milk. The mixtures were heated to 85°C for 5 min to denature the protein and entrap water. Viscosity was increased with as little as .6% added whey protein. A custard-like gel with sufficient body to stand alone without leakage was formed with 1.5% whey protein added to the skim milk. About twice as much egg albumin was required for similar results. A 10% solution of WPC with 50% of the solids as protein gave a firm gel with no leakage after heat treatment (44). Protein gelation may be effectively utilized to enhance

functional properties in a variety of food systems (56).

Replacement Whey Solids in Yogurt

Whey solids can be used advantageously in the manufacture of particular cultured milk products providing all the milk constituents are retained in the finished product. The amount of whey solids used in the manufacture of cultured products has not been developed to the same extent as its usage in other food products. The reported applications are still experimental and have not yet attained commercial status (51). Jelen and Horbal (32) prepared various mixtures of liquid cottage cheese whey with or without fresh homogenized milk for use in reconstituting NDM for yogurt manufacture. Using commercial yogurt cultures with incubation at 45$^{o}$C for 4 to 6 h, yogurts were made with the different formulations of cottage cheese whey. Results obtained with a penetrometer showed an increase in firmness with increasing total solids and increased proportion of homogenized milk. Satisfactory plain and flavored yogurts were made from 60% cottage cheese whey, 29% homogenized milk, and 11% NDM.

Todoric and Savadinovic (62) added varying amounts of dry whey from .2 to .6% to a pasteurized yogurt base containing 3.2% fat. Milk containing .2% added NDM served as the control. After addition of the NDM or dried whey, the milk was repasteurized at 82$^{o}$C/15 min, homogenized at 200 atm, inoculated with 2% culture, and incubated at 42$^{o}$C for 3 h. Results of the organoleptic evaluations showed samples with .4% dried whey lacked specific aroma, had a pronounced sweet off-flavor,

and sustained separate whey after storage. On the basis of another experiment, it was concluded that a maximum of .3% whey could be used in place of NDM in yogurt manufacture.

Griffen (24) formulated yogurts using liquid and powdered WPC's in the various formulations to partially or wholly replace NDM. Whole milk plus 5% NDM served as the control. Formulations employing either liquid or powdered WPC to replace one half of the NDM were found to be either equal or superior in flavor to the control and to commercially produced yogurts. Formulations with one half WPC and one half NDM showed no defects in body after 5 days storage and transport.

Hartman (29) formulated yogurt with sweet, acid, and modified dry wheys and neutralized liquid cottage cheese whey to supply added milk solids. A whey off-flavor was detected in yogurts containing 3% or more whey solids supplied by neutralized fluid cottage cheese whey. A slight whey off-flavor was detected in plain yogurt containing 2% whey solids but this flavor could not be detected in strawberry flavored Swiss style yogurt. Yogurts made from dry sweet whey, dry acid whey, and concentrated acid whey were found to be about the same in flavor, body characteristics, and color as yogurts made with fresh neutralized whey. Yogurts made with different types of modified dry wheys showed an improvement or were at least equivalent in body characteristics with increasing levels of added whey solids. A whey off-flavor was apparent at the 3% replacement level and was very slight at the 2% level in plain yogurt. No off-flavor could be

detected in the strawberry flavored Swiss style samples. It was concluded sweet whey solids or neutralized cottage cheese whey solids could be used in yogurt manufacture at the rate of 1 to 2% to replace an equivalent amount of NDM without affecting body, providing total milk solids-not-fat were at least 9.5%. Federal regulations promulgated by the Food and Drug Administration (FDA) permit the following materials to be added to yogurt as optional ingredients: milk powder, skimmed milk powder, unfermented buttermilk, concentrated whey, whey powder, whey proteins, whey protein concentrate, water-soluble milk proteins, edible casein, and caseinates; all manufactured from pasteurized products (20).

## Lactose Hydrolysis

The second aspect of this project involved enzymatic hydrolysis of the lactose in the yogurt mix prior to culturing. Research on the application of lactose hydrolysis in dairy products has become more popular in recent years as food and dairy processors become more aware of the gastrointestinal problems suffered by lactose intolerant and lactose sensitive individuals. Lactose intolerant persons either do not have lactase enzyme or do not have sufficient amounts of lactase in the intestinal wall (55). Lactose must undergo hydrolysis into its component monosaccharides, glucose and galactose, which are readily absorbed from the intestines, before it can be metabolized by the body. If not hydrolyzed by lactase, the lactose is not absorbed but will remain in the intestinal lumen and act osmotically

to draw water into the small intestine. This outpouring fluid into the jejunum produces bloating, cramping, and diarrhea. Symptoms usually appear .5 to 3 h after ingestion of one to three glasses of milk. In the U.S.A., about 10 to 15% of adult Caucasians and about 70% of adult Blacks are afflicted with this condition (55).

Lactase Enzyme

Lactase is the enzyme β-galactosidase (EC.3.2.1.23) which catalyzes the hydrolysis of the 1, 4, β-linkage of lactose liberating one mole of D-glucose and one mole of D-galactose. Lactase enzyme is secreted by many microorganisms and has been described in *Aeromonas formicans*, *Shigella soneii*, *Escherichia coli*, *Bacillus subtilis*, *Kluyveromyces fragilis*, *Neurospora crassa*, *Aspergillus niger*, and others (63).

Since the early 1950's, the potential for enzymatic modification of lactose in dairy products has been recognized but was not possible with more than small laboratory amounts until the recent development of commercial processes for the isolation of the enzyme from microbial sources. The β-galactosidase isolated from *Aspergillus niger* and *Saccharomyces lactis* have the most desirable functional characteristics suitable for commercial use in dairy products. These enzymes differ greatly in their properties, especially in pH optima. β-Galactosidase isolated from *A. niger* has a pH optimum of 4.0 to 4.5 and would therefore be limited to use in products containing acid whey. The β-galactosidase from *S. lactis* has a more favorable pH

optimum (6.5 to 7.0) and pH stability range (6.0 to 8.5) for lactose hydrolysis in milk products and sweet whey products (31).

Enzymatic hydrolysis of lactose may be achieved via two methods: addition of free enzyme directly to the substrate or by immobilization of the enzyme (binding it to a solid carrier) and then passing the substrate over it. While adding the enzyme preparation directly to the medium is the simplest method, it is the most expensive since the enzyme is not recoverable for further use (31). Enzyme immobilization is the most economical method with probable extended usage of the bound enzymes, which can reduce costs significantly. β-Galactosidase isolated from A. niger is more suitable for use in immobilized systems than yeast or bacterial lactase. In any large scale lactose hydrolysis process, the purity, availability, and cost of β-galactosidase are important considerations (67).

## Commercial Applications of Lactose Hydrolysis

Not only would lactose hydrolysis allow a higher consumption of milk by lactose intolerant individuals, but lactose-hydrolyzed products can be used to greater advantage in many commercial applications. Lactose is the main barrier to full utilization of whey in food products because the low solubility of lactose causes a grainy texture in foods. Many of the problems encountered when adding whey to food products would be solved by hydrolysis of the lactose into glucose and galactose. The resulting sugar mixture is more soluble,

sweeter, more easily digestible, and is more readily fermented by a greater number of organisms (30, 31, 52). Glucose and galactose are more soluble and sweeter than lactose itself, which would be advantageous in the production of ice creams, frozen concentrates, and in lactose-derived syrups. Hydrolysis also prevents lactose crystallization (31). Tests using a partially hydrolyzed and demineralized whey as a replacement for up to 25% of skimmilk and sugar in ice cream, produced an ice cream more resistant to crystallization and sandiness. The ice cream was also sweeter, had a better body, improved mouthfeel, and a softer texture. Candies and confections made with hydrolyzed whey demonstrated good caramelization, additional sweetness, softer textures, and no graininess (3).

Lactose Hydrolysis in Yogurt

Engle (17) formulated a yogurt mix with 4% NDM to increase the solids content and added three different amounts of MAXILACT® lactase to equal portions of the yogurt mix. The finished yogurt samples were organoleptically evaluated by two groups. The first group consisted of those persons who admittedly did not like the "sour milk" taste of plain yogurt and the second group consisted of those who like plain yogurt. The average acceptance level for the first group was about 40% lactose hydrolysis, and rejection was at 60% lactose hydrolysis among those who like yogurt. Engle concluded yogurt with 50% lactose hydrolysis should be acceptable to both groups. Thompson and Gyuricsek (61) noted a reduction in the incubation time required to

reach a desired pH when preparing yogurts from lactose-hydrolyzed (LH) milks. Yogurts prepared from 90 to 95% LH milks were found to be sweeter than the controls and had a more acceptable flavor to persons who did not normally eat yogurt.

In another study, Gyuricsek and Thompson (27) prepared yogurts from 0, 25, 50, 75, and >90% LH yogurt mixes fortified with 4% NDM. The incubation time required to reach the desired pH values of 4.6 was reduced by 40 min and the high acid flavor was found to be partially off-set by the sweetness imparted by the glucose and galactose. Yogurts prepared from hydrolyzed milks were preferred in a comparative evaluation with plain yogurt and were also smoother in body than the control. It was suggested that consumption of LH yogurt would reduce the lactose intolerance reaction, improve the overall nutrition of the consumer, and result in increased sales of the product (27).

Yogurts were prepared from fortified control and hydrolyzed lactose (70-75%) milks by O'Leary and Woychik (53). Faster acid development was noted in the LH yogurt samples. Flavor evaluations of the yogurts by a sensory panel showed a significantly higher preference for the LH yogurt compared to the control products. Preference was attributed to the sweeter character imparted by the free glucose and galactose. Some panel members detected some other flavor differences between the two yogurts in addition to sweetness; but the substantially greater sweetness of the LH yogurt was the major factor in the flavor evaluations. The authors concluded that manufacture of cultured dairy products from LH milks may result in changes of the

profiles of the resulting product.

Faster acid development (or a shorter coagulation time) in LH yogurts was also observed by Antila et al. (5). Yogurts were prepared from control milks and from lactase-treated milks with 24, 40, and 64% hydrolysis. Organoleptic evaluators preferred the taste of the LH yogurts to the taste of the control. Hilgendorf (30) observed a shorter coagulation time for yogurts manufactured with lactase in the mix. Various levels of fungal lactase were pipetted into containers of yogurt mix excepting the control. Containers were covered and yogurts incubated until coagulation occurred. The decrease in set time varied with the amount of lactase added and the percentage of culture inoculum. The degree of hydrolysis varied with the amount of lactase added. Yogurts made with 50 to 300 mg/liter added lactase, representing 20 to 40% hydrolysis, were preferred by tasters. Too much lactase was presumed to result in an intensely sweet product with a bitter after taste. Organoleptic preference for yogurts made with fungal lactase was found to be similar to that of yogurts made from lactase treated milk hydrolyzed before manufacture. It was concluded that the use of fungal lactase in yogurt manufacture is a natural means of improving product quality and acceptability through increased sweetness without adding calories.

# MATERIALS AND METHODS

Yogurt mixes were formulated from 2% lowfat milk with added milk serum solids for improved body, granulated sugar[1] for sweetness, and a stabilizer. Four percent nonfat dry milk[2] (NDM) to fortify serum solids and 4% sucrose were chosen as representative of average commercial values, and were used in the control lots to which the experimental formulations were compared. Melotein®,[3] WM-34 and Melotein®,[3] MP-34 were chosen as economical replacements for NDM. These products, WM-34 and MP-34, are spray dried reconstructed milk products (RMP) with high concentrations of milk proteins and are manufactured from sweet whey and caseinate blends. These RMP's are specially formulated to match the functional, chemical, and nutritional properties of NDM. The compositions and comparative costs of these products and NDM are given in Table 1. Gelatin[4], at the rate of .5%, was chosen as the stabilizer. No fruit or flavorings were added, so any inherent flavor differences could be organoleptically detected.

Fifty or one hundred percent of the 4% added NDM was replaced in the experimental yogurt mixes by one or the other of the RMP's; thus, there were a total of five different formulations. Lactose was partially hydrolyzed enzymatically to provide additional sweetness so the amount of sucrose could be reduced to lower calories and cost.

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[1] Amalgamated Sugar Company, Ogden, UT 84401.

[2] Land O'Lakes, Inc., P.O. Box 116, Minneapolis, MN 55440.

[3] Dairyland Products, Inc., 5345 W. 125 Street, Savage, MN 55378.

[4] Swift & Company, 1215 Harrison Avenue, Kearny, NJ 29140.

TABLE 1. Composition[a] and cost[a] of NDM[b], WM-34[c], and MP-34[c] used in manufacture of yogurt.

| | NDM | WM-34 | MP-34 |
|---|---|---|---|
| Total solids (%) | 96.5 | 96.1 | 96.4 |
| Protein (%) | 33.5 | 34.8 | 35.2 |
| Lactose (%) | 54.9 | 51.6 | 52.0 |
| Fat (%) | 1.1 | 2.5 | 1.1 |
| Ash (%) | 8.0 | 7.2 | 8.1 |
| Cost ($/kg) | 2.42[a] | 1.43[a] | 1.48[a] |

[a]Values given by product suppliers.
[b]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.
[c]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

Within each formulation, three mixes were made: one with no hydrolysis, one with 50% of the lactose hydrolyzed, and one with 75% of the lactose hydrolyzed. The sucrose level in the 4% WM-34 and 4% MP-34 mixes (0% hydrolysis) was 2%. The sucrose level was reduced another 1% in all mixes with 50 or 75% hydrolysis to allow for the additional sweetness produced by hydrolysis of the lactose to the component monosaccharides. Since there is little casein in the RMP's to mask sweetness (43), the 4% MP-34 and 4% WM-34 formulations with 4% sucrose were much sweeter than the 4% NDM formulation with 4% sucrose. Experimental reductions of the sucrose level were conducted and a level of 2% sucrose was found to impart the same degree of sweetness as the 4% NDM formulation with 4% sucrose. Fifteen yogurt mixes comprised one experimental series which were replicated five times. The fifteen formulations are given in Table 2.

Pasteurized, homogenized 2% lowfat milk was obtained from the South Dakota State University (SDSU) Dairy Products Laboratory. All yogurt mixes were formulated from 1.89 liters (.5 gal) of milk; the added NDM, RMP's, sucrose, and stabilizer were calculated on a weight basis. The milk was measured into a 3 liter Erlenmeyer flask and the dry ingredients were weighed ($\pm$.1 g), quantitatively transferred, and blended into the milk by gently stirring with a magnetic bar. The mixture was continuously stirred while being heated to 85$^{o}$C and held for 30 min to effectuate pasteurization. The nonhydrolyzed yogurt mixes were cooled to 44 to 45$^{o}$C after pasteurization, inoculated with 2% by volume of each of two 14 to 15 h cultures, Streptococcus

TABLE 2. Formulations of the fifteen yogurt mixes.

| Ingredients | No lactose hydrolysis | 50% lactose hydrolysis | 75% lactose hydrolysis |
|---|---|---|---|
| | No replacement of NDM[a] | | |
| | (%) | | |
| NDM[a] | 4 | 4 | 4 |
| Sucrose | 4 | 3 | 3 |
| Gelatin | .5 | .5 | .5 |
| | 50% replacement of NDM[a] by WM-34[b] | | |
| | (%) | | |
| NDM[a] | 2 | 2 | 2 |
| WM-34[b] | 2 | 2 | 2 |
| Sucrose | 3 | 2 | 2 |
| Gelatin | .5 | .5 | .5 |
| | 100% replacement of NDM[a] by WM-34[b] | | |
| | (%) | | |
| WM-34[b] | 4 | 4 | 4 |
| Sucrose | 2 | 1 | 1 |
| Gelatin | .5 | .5 | .5 |
| | 50% replacement of NDM[a] by MP-34[b] | | |
| | (%) | | |
| NDM[a] | 2 | 2 | 2 |
| MP-34[b] | 2 | 2 | 2 |
| Sucrose | 3 | 2 | 2 |
| Gelatin | .5 | .5 | .5 |
| | 100% replacement of NDM[a] by MP-34[b] | | |
| | (%) | | |
| MP-34[b] | 4 | 4 | 4 |
| Sucrose | 2 | 1 | 1 |
| Gelatin | .5 | .5 | .5 |

[a]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[b]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

thermophilus and Lactobacillus bulgaricus, transferred into 500 ml plastic containers, and incubated at 42°C until the proper titratable acidity (TA), expressed as percent lactic acid, was reached.

The enzymatically hydrolyzed mixes were blended and pasteurized as previously outlined, but after pasteurization were cooled to 32°C and MAXILACT®,[1] LX5000 (5000 Neutral Lactase Units/g) β-galactosidase added at the rate of .29 ml/liter of mix. The mixes were continuously stirred and maintained at 30 to 32°C for 65 min for the 50% hydrolyzed or 2 h for the 75% hydrolyzed mixes. After hydrolysis, the mixtures were heated to 70°C for 2 min to denature the enzyme, cooled to 44 to 45°C and inoculated, transferred into 500 ml plastic containers, and incubated at 42°C. The initial TA was measured after inoculation and one carton was periodically sampled and titrated for TA until a value of .96 to .98% was reached. All cartons were then carefully transferred to a cooler adjusted to 5°C where a final TA of approximately 1.05% would be reached. A flow diagram of the manufacturing steps is presented in Figure 1.

This study utilized the lactase enzyme preparation known commercially as MAXILACT®, produced by the yeast Saccharomyces lactis. MAXILACT® is considered a "generally regarded as safe" (GRAS) substance based on the following:

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[1]GB Fermentation Industries, Inc., 5550 - 77 Center Drive, P.O. Box 241068, Charlotte, NC 28224.

Figure 1. Flow diagram of yogurt manufacture.

2% lowfat milk in 3 liter Erlenmeyer flask

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Add milk or whey solids, sucrose, stabilizer

↓

Pasteurize mix at 85°C for 30 min

**Nonhydrolyzed mixes**

↓

Cool to 44 to 45°C

↓

Inoculate with 2% cultures

↓

Transfer to 500 ml containers and incubate at 42°C until TA reached .96 to .98%.

↓

Refrigerate at 5°C

↓

Compositional analyses and organoleptic evaluations

**Hydrolyzed mixes**

↓

Cool to 32°C

↓

Add appropriate amount of enzyme and hold

↓

Denature enzyme at 70°C for 2 min

↓

Cool to 44 to 45°C

↓

Inoculate with 2% culture

↓

Transfer to 500 ml containers and incubate at 42°C until TA reached .96 to .98%

↓

Refrigerate at 5°C

↓

Compositional analyses and organoleptic evaluations

(1) Petition for affirmation of GRAS status filed by the Ad Hoc Enzyme Technical Committee on April 11, 1973 (18).
(2) This petition places carbohydrases from Saccharomyces species in the same catagory as the widely used food enzymes produced by Bacillus subtilis, Aspergillus niger, and Aspergillus oryzae (19).

The yogurt cultures used were transfers of reconstituted freeze dried Hansen's[1] yogurt cultures. Streptococcus thermophilus and Lactobacillus bulgaricus were separately propagated and maintained in sterilized reconstituted NDM (113 g/liter) at 38°C and 45°C, respectively. The cultures were transferred daily during yogurt manufacturing with 1% inocula (1 ml culture in 99 ml milk).

## Sampling

Mix samples were taken after pasteurization but before culture inoculation for nonhydrolyzed formulations, and before and after hydrolysis for hydrolyzed formulations. Mix samples were preserved by freezing (-18°C) in plastic sample bottles until time of compositional analyses. One carton of each finished yogurt was frozen (-30°C) for selected compositional analyses.

## Compositional Analyses

The following compositional tests were run on uncultured nonhydrolyzed mix samples and on samples taken from hydrolyzed mixes

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[1]Chr. Hansen's Laboratory, Inc., 9015 West Maple Street, Milwaukee, WI 53214.

before the addition of lactase. Fat and total solids (TS) contents of the yogurt mixes were determined by the Mojonnier procedures (46). The Kjeldahl total protein procedure for milk from the Association of Official Analytical Chemists (AOAC) (6) was used to ascertain the protein in all mix samples. Total ash content was determined by the AOAC (6) procedure for ash in milk using porcelain crucibles. The initial lactose content was determined by the method described by Nickerson et al. (49). Nonhydrolyzed samples were diluted to the total factor of 50 as described under preparation of sample. Hydrolyzed samples were diluted to a factor of 12.5 in order to keep the spectrophotometric transmittance values between 65 and 15%. Lactose determinations were also performed on uncultured mix samples taken after the denaturation of the enzyme in order to determine the exact percentage of hydrolysis.

Titratable acidity (TA) (2) expressed as percent lactic acid was measured using a Nafis Automatic Acidity Test bottle on finished yogurts 24 h after termination of incubation and again after 1 wk and 2 wk storage. Using a Corning pH meter Model 7, pH values were measured 24 h after incubation, and after 1 wk and 2 wk storage at $5^{o}C$. Final lactose contents in all finished yogurts were determined following the method described by Nickerson et al. (49) reducing the dilution factor to 12.5.

Direct microscopic counts (DMC) stained smears were prepared on all finished yogurt samples according to the method described by the Standard Methods for the Examination of Dairy Products (2) in

order to determine the ratio of cocci to rods. Yogurt samples were diluted 1:100 and applied to the slide in portions larger than the specified .01 ml test portion because an exact count was not needed, only the ratio of cocci to rods. The calories per 100 g of yogurt were calculated for each sample according to the equation: cal = [(% fat x 9) + {% TS - (% fat + .7%} x 4] (37).

All finished yogurt samples were analyzed for the presence of acetaldehyde (8), an important flavor compound in yogurt. In the presence of aldehyde dehydrogenase (AL-DH), acetaldehyde is oxidized by nicotinamide-adenine dinucleotide (NAD) to acetic acid. The amount of NADH formed is stoichiometric with the amount of acetaldehyde and is determined by means of its absorption at 334, 340, or 365 nm. In glass cuvettes, one for the blank and one for the sample, 1 ml of buffer was added (5.0 g $K_4P_2O_7$ in 40 ml redist. $H_2O$, adjusted to pH 9 with 1 mol/liter HCl; filled to 50 ml with redist. $H_2O$). To both cuvettes .10 ml NAD solution was added (110 mg NAD with 3 ml redist. $H_2O$; stable for 4 wk at $4^oC$). Then 2 ml redist. $H_2O$ was mixed in the cuvette for the blank. A .10 ml sample was mixed with 1.90 ml redist. $H_2O$ in the sample cuvette, and the optical density ($E_1$) of both cuvettes read after 2 to 3 min on a double beam spectrophotometer. The reaction was started by the addition of .02 ml AL-DH (40 U AL-DH with 5 ml redist. $H_2O$; stable for 8 h at $4^oC$) to both cuvettes, mixed, and after 3 to 4 min the optical densities ($E_2$) were read. The cuvettes must be stoppered and be read against air or water. The optical density differences ($E_2$-$E_1$) were determined for both blank and

sample. The optical density difference of the blank ($\Delta E_B$) was subtracted from the optical density differences of the sample ($\Delta E_S$); a positive difference indicates the presence of acetaldehyde.

Organoleptic Evaluation

The finished yogurts were organoleptically evaluated by two methods: with a panel consisting of Dairy Science faculty and a consumer panel. Samples were evaluated 1 to 2 days after manufacture of the last batch in the experimental lot. A set of yogurts for a taste test included the three NDM formulations and the 50% replacement and 100% replacement formulations were tasted along with the NDM formulations. Nine yogurt samples were tasted at one time and were presented randomly to the panel in order to prevent indentification of samples. The Dairy Science faculty panel consisted of five judges evaluating the yogurts for flavor defects using the American Dairy Science Association-Dairy Food Industry Supply Association (ADSA-DFISA) yogurt score card. An example of the ADSA yogurt score card is given in Figure 2. University students were randomly recruited for the consumer taste panel which consisted of at least 25 students for each taste test. Each student was given nine yogurt samples and asked to rate the sample on a nine point hedonic scale (9=like extremely; 1=dislike extremely). An example of the consumer survey score card is illustrated in Figure 3.

Figure 2. American Dairy Science Association product judging score card for Swiss style yogurt.

SWISS STYLE YOGURT SCORE CARD

FLAVOR:
DATE ____________ D.F.I.S.A. ______ CONTESTANT NO.______

| Perfect Score | Criticisms | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | TOTAL GRADE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Flavor - 10 | Contestant Score | | | | | | | | | | | |
| | Grade Score | | | | | | | | | | | |
| | Grade Criticism | | | | | | | | | | | |
| No Criticism 10 | High Acid | | | | | | | | | | | |
| | Low Acid | | | | | | | | | | | |
| | Bitter | | | | | | | | | | | |
| | Cooked | | | | | | | | | | | |
| | Green | | | | | | | | | | | |
| | Lacks Fine Flavor | | | | | | | | | | | |
| | Lacks Flavor | | | | | | | | | | | |
| | Lacks Freshness | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | Lacks Sweetness | | | | | | | | | | | |
| | Old Ingredient | | | | | | | | | | | |
| | Oxidized | | | | | | | | | | | |
| | Rancid | | | | | | | | | | | |
| | Stabilizer | | | | | | | | | | | |
| | Too High Flavor | | | | | | | | | | | |
| | Too Sweet | | | | | | | | | | | |
| | Unnatural Flavor | | | | | | | | | | | |
| | Unclean | | | | | | | | | | | |
| Body & Texture - 5 | Contestant Score | | | | | | | | | | | |
| | Grade Score | | | | | | | | | | | |
| | Grade Criticism | | | | | | | | | | | |
| | Gel-like | | | | | | | | | | | |
| | Grainy | | | | | | | | | | | |
| | Lumpy | | | | | | | | | | | |
| | Ropy | | | | | | | | | | | |
| | Too Firm | | | | | | | | | | | |
| | Weak | | | | | | | | | | | |
| Appearance 5 | Contestant Score | | | | | | | | | | | |
| | Grade Score | | | | | | | | | | | |
| No Criticism 5 | Grade Criticism | | | | | | | | | | | |
| | Atypical Color | | | | | | | | | | | |
| | Excessive Fruit | | | | | | | | | | | |
| | Lacks Fruit | | | | | | | | | | | |
| Normal Range 1-5 | Free Whey | | | | | | | | | | | |
| | Shrunken | | | | | | | | | | | |
| | Surface Growth | | | | | | | | | | | |
| Total | Total Score of Each Sample | | | | | | | | | | | |
| | TOTAL GRADE PER SAMPLE | | | | | | | | | | | |

FINAL GRADE ______

RANK ______

TEAM RANK

| | Code | Grade |
|---|---|---|
| | 1 | |
| | 2 | |
| | 3 | |
| TOTAL | | |
| RANK | | |

Figure 3. Sample consumer survey score card.

| Sample number | Like extremely 9 | Like very much 8 | Like moderately 7 | Like slightly 6 | Neither like or dislike 5 | Dislike slightly 4 | Dislike moderately 3 | Dislike very much 2 | Dislike extremely 1 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | |
| 2 | | | | | | | | | |
| 3 | | | | | | | | | |
| 4 | | | | | | | | | |
| 5 | | | | | | | | | |
| 6 | | | | | | | | | |
| 7 | | | | | | | | | |
| 8 | | | | | | | | | |
| 9 | | | | | | | | | |

Statistical Analysis

Statistical analysis of the data utilized the analysis of variance for a factorial experiment with a three factor (formulation, hydrolysis, and replication) design (59). The main effects of formulation and hydrolysis were tested by the respective main effect and replication interaction. The Waller-Duncan k-Ratio t Test was employed to determine the differences between data means (59).

# RESULTS AND DISCUSSION

## Yogurt Mix and Final Yogurt Composition

Nonfat dry milk (NDM) and two reconstructed milk products (RMP) were used singly or as equal parts NDM and one of the RMP's to fortify 2% lowfat milk for the manufacture of yogurt. Lactose in all the compounded mixes was enzymatically hydrolyzed by 0, 50, or 75% before fermentation. Since the RMP's were specially formulated to match the composition of NDM, all yogurts should have been similar with respect to fat, protein, ash, and initial lactose. The total solids contents should have varied as different levels of sucrose were added to adjust for sweetness imparted from the products of lactose hydrolysis. Table 3 shows the average protein, fat, total solids, ash, and initial lactose contents of the yogurt mixes.

Analysis of variance was performed on the protein contents in the yogurt mixes. The results as shown in Table 4 detected no differences ($P<.05$) among formulations, levels of lactose hydrolysis, or the interaction of the two factors. This was the expected result since the protein contents of the added NDM and RMP's were similar (Table 1). The protein contents in the yogurt mixes (means of five replications) are presented in Table 3 and ranged from 4.90 to 4.99% with an overall mean of 4.94%.

The percentages of fat in the mixes before fermentation were found to be different ($P<.05$) among the nonhydrolyzed and hydrolyzed yogurts and, also within the interaction of hydrolysis and formulation as seen in Table 5. The differences detected in the hydrolysis factor

TABLE 3. Composition of five yogurt formulations with 0, 50, or 75% hydrolysis of lactose[a].

| Component | 4% NDM[b] | | | 2% WM-34[c] 2% NDM[b] | | | 4% WM-34[c] | | | 2% MP-34[c] 2% NDM[b] | | | 4% MP-34[c] | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 75 | 0 | 50 | 75 | 0 | 50 | 75 | 0 | 50 | 75 | 0 | 50 | 75 | |
| | (%) | | | | | | | | | | | | | | | |
| Protein | 4.90 | 4.96 | 4.94 | 4.94 | 4.91 | 4.94 | 4.98 | 4.99 | 4.92 | 4.91 | 4.97 | 4.95 | 4.90 | 4.97 | 4.98 | 4.94 |
| Fat | 1.84 | 1.89 | 1.94 | 1.91 | 1.95 | 1.90 | 1.87 | 1.91 | 1.97 | 1.96 | 1.93 | 1.95 | 1.82 | 1.96 | 1.94 | 1.92 |
| Total Solids | 17.99 | 17.20 | 17.17 | 17.14 | 16.45 | 16.46 | 16.36 | 15.47 | 15.48 | 17.13 | 16.36 | 16.39 | 16.46 | 15.38 | 15.53 | 16.47 |
| Ash | 1.02 | .99 | 1.02 | 1.02 | 1.03 | 1.01 | 1.02 | 1.03 | 1.02 | 1.01 | 1.02 | 1.02 | 1.02 | 1.04 | 1.02 | 1.02 |
| Initial lactose | 6.92 | 6.94 | 6.95 | 6.97 | 6.99 | 7.00 | 7.00 | 6.99 | 7.04 | 6.97 | 6.95 | 6.98 | 6.95 | 7.01 | 7.02 | 6.98 |

[a]Values are means of five replications (tests done in duplicate).

[b]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[c]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

TABLE 4. Analysis of variance[a] of protein percent in five yogurt formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .008 | |
| Rep | 4 | .048 | |
| Form | 4 | .003 | .49[NS] |
| Rep x Form | 16 | .006 | |
| Hyd | 2 | .006 | 1.16[NS] |
| Rep x Hyd | 8 | .005 | |
| Form x Hyd | 8 | .005 | .79[NS] |
| Rep x Form x Hyd | 32 | .006 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

TABLE 5. Analysis of variance[a] of fat percent in five yogurt formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .006 | |
| Rep | 4 | .025 | |
| Form | 4 | .008 | 1.26[NS] |
| Rep x Form | 16 | .006 | |
| Hyd | 2 | .024 | 7.28* |
| Rep x Hyd | 8 | .003 | |
| Form x Hyd | 8 | .008 | 2.28* |
| Rep x Form x Hyd | 32 | .004 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

*Significant (P<.05).

were between the nonhydrolyzed yogurts and the hydrolyzed yogurts. The means of the fat contents in the 50 and 75% levels of lactose hydrolysis were similar to each other. These differences in fat content could be explained by the variation of the milk-solids-not fat ingredients in the various mix formulations or by the dilution effect created by the varying sucrose contents in the respective formulations. The fat percentages in the mixes are summarized in Table 3 and varied from 1.82 to 1.97% with an overall mean of 1.92%. Federal standards (20) specify that lowfat yogurt must contain not less than .5% nor more than 2% milk fat, so all formulations complied with the Federal Standards of Identity.

Total solids in the mixes included solids from the 2% lowfat milk plus the added NDM and RMP solids and the solids of non-milk origin (sucrose and gelatin). The percentages of total solids in all yogurt mixes (means of five series of yogurts) are presented in Table 3. Table 6 contains the results of the statistical analysis which detected a difference ($P<.01$) in the total solids means among the nonhydrolyzed and hydrolyzed mixes and also among the various formulations. The interaction of formulation and hydrolysis was also significant. The differences among the five formulations can be summarized as follows: yogurts containing 4% NDM varied from the remaining four; those yogurts containing 2% WM-34 or 2% MP-34 were similar to each other in TS content but not to the other formulations; and those manufactured with 4% WM-34 or 4% MP-34 were similar to each other but not to the rest of the formulations. This was the expected result since

TABLE 6. Analysis of variance[a] of total solids percent in five yogurt formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .568 | |
| Rep | 4 | .017 | |
| Form | 4 | 7.430 | 742.02** |
| Rep x Form | 16 | .010 | |
| Hyd | 2 | 5.713 | 655.20** |
| Rep x Hyd | 8 | .009 | |
| Form x Hyd | 8 | .032 | 2.81* |
| Rep x Form x Hyd | 32 | .011 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

** Highly significant (P<.01).

*Significant (P<.05).

the 4% NDM (no hydrolysis) formulation contained 4% sucrose; the 2% WM-34 and 2% MP-34 (no hydrolysis) formulations contained 3% sucrose; and the 4% WM-34 and 4% MP-34 formulations (no hydrolysis) contained 2% sucrose. The 50 and 75% hydrolyzed mixes were similar in total solids content but both varied from the nonhydrolyzed mixes in total solids content. This phenomenon was also expected since in any formulation the sucrose content in all 50 and 75% hydrolyzed mixes was reduced 1% from the corresponding nonhydrolyzed mixes (Table 2).

The ash content values as seen in Table 7 indicated no differences ($P>.05$) among the yogurt mixes. Since the percentages of ash in the RMP's and NDM (Table 1) were similar and other constituents were common to all mixes, no real differences in ash contents of yogurt mixes were expected. The percentages of ash (mean of five replications) are presented in Table 3 and varied from .99 to 1.04%.

Initial lactose contents in the yogurt mixes differed ($P<.05$) among the five formulations, among the three hydrolysis levels ($P<.05$), but not among the interaction of the formulations and three hydrolysis levels factors ($P>.05$). Table 8 tabulates the results of this analysis. Differences in lactose content were found between the 4% NDM formulation and the other four formulations. The lactose contents of the 2% WM-34, 4% WM-34, 2% MP-34, and 4% MP-34 mixes were all similar to each other. The lactose contents of the mixes varied between the 0% and the 75% hydrolyzed mixes. The percentages of lactose in the 50% hydrolyzed mixes were similar in content to both the 0 and 75% hydrolyzed mixes. The variations in lactose content in

TABLE 7. Analysis of variance[a] of ash percent in five yogurt formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .001 | |
| Rep | 4 | .003 | |
| Form | 4 | .001 | 1.47[NS] |
| Rep x Form | 16 | .0004 | |
| Hyd | 2 | .0001 | .64[NS] |
| Rep x Hyd | 8 | .0001 | |
| Form x Hyd | 8 | .001 | 1.13[NS] |
| Rep x Form x Hyd | 32 | .001 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

TABLE 8. Analysis of variance[a] of initial lactose percent in five yogurt formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .003 | |
| Rep | 4 | .006 | |
| Form | 4 | .012 | 4.59* |
| Rep x Form | 16 | .003 | |
| Hyd | 2 | .008 | 5.49* |
| Rep x Hyd | 8 | .002 | |
| Form x Hyd | 8 | .002 | .82[NS] |
| Rep x Form x Hyd | 32 | .002 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

*Significant ($P<.05$).

[NS]Not significant.

the mixes may have been a result of the varying dilution effects caused by the changes in sucrose content from formulation to formulation or within the three levels of hydrolysis. The lactose content may also have varied as a result of the lactose content of the NDM (54.9%), WM-34 (51.6%), and MP-34 (52.0%). The overall initial lactose values in the yogurt mixes ranged from 6.92 to 7.04% with a mean of 6.98% (Table 3).

Titratable acidities expressed as percent lactic acid are shown in Table 9 and were recorded at the end of incubation, after 24 h, after 1 wk, and after 2 wk storage at 5$^{o}$C. After termination of incubation, the cultures would continue to multiply and produce lactic acid until the yogurts were cooled to temperatures below their viable growth ranges. The TA would be expected to rise during storage periods since the organisms were still viable and capable of producing acid and, also since during measurement of TA, the yogurts partially warmed to room temperature. Flückiger and Walser (21) noted a rise in titratable acidities in plain and apricot-flavored yogurts during storage at 5 and 15$^{o}$C. Titratable acidities after 1 wk and 2 wk storage (5$^{o}$C) were recorded for the 3rd, 4th, and 5th set of replicates only. An increase in TA was noted 24 h after manufacture, after 1 wk storage, and after 2 wk storage. Titratable acidities showed no differences ($P>.05$) among the values recorded 24 h after manufacture (Table 10), after 1 wk storage (Table 11), or after 2 wk storage (Table 12) among the five formulations or the three hydrolysis levels.

TABLE 9. Titratable acidities as percent lactic acid of yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Time | 4% NDM[a] | | | 2% WM-34[b] 2% NDM[a] | | | 4% WM-34[b] | | | 2% MP-34[b] 2% NDM[a] | | | 4% MP-34[b] | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 75 | 0 | 50 | 75 | 0 | 50 | 75 | 0 | 50 | 75 | 0 | 50 | 75 | |
| | (%) | | | | | | | | | | | | | | | |
| End of incubation[c] | .97 | .96 | .99 | .97 | .96 | .97 | .98 | .96 | .98 | .97 | .97 | .98 | .97 | .98 | .96 | .97 |
| After 24 h[c] | 1.07 | 1.06 | 1.05 | 1.04 | 1.03 | 1.08 | 1.09 | 1.06 | 1.06 | 1.04 | 1.04 | 1.06 | 1.09 | 1.07 | 1.05 | 1.06 |
| After 1 wk[d] | 1.11 | 1.10 | 1.12 | 1.10 | 1.12 | 1.12 | 1.13 | 1.13 | 1.16 | 1.11 | 1.14 | 1.11 | 1.15 | 1.14 | 1.11 | 1.12 |
| After 2 wk[d] | 1.22 | 1.16 | 1.16 | 1.18 | 1.16 | 1.17 | 1.17 | 1.20 | 1.23 | 1.18 | 1.17 | 1.15 | 1.27 | 1.19 | 1.19 | 1.19 |

[a]Nonfat dry milk, Land O'Lakes, Inc, Minneapolis, MN.

[b]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

[c]Values are means of five replications.

[d]Values are means of the last three replications.

The analysis of variance was performed only on TA's recorded during each time period (after 24 h, after 1 wk, after 2 wk storage), not on the increase in TA values between each time period. Thus, it is not known whether the rises in TA during storage were significantly different. Federal standards (20) state that yogurt, lowfat yogurt, and nonfat yogurt must have a TA of not less than .9%, expressed as lactic acid; all yogurts were well above .9% TA.

The pH's of all yogurts were measured 24 h after manufacture, after 1 wk storage at 5°C, and after 2 wk storage at 5°C and are contained in Table 13. The latter two measurements were made on the 3rd, 4th, and 5th set of replicates only. Since the literature (21) predicts a rise in TA values during storage, pH values would be expected to drop even at storage temperatures. A decrease was noted after 1 wk storage and again after 2 wk storage. Again, the statistical analysis was performed on the values within each time period, not values from one time period to the next. No differences ($P>.05$) were detected in pH values recorded 24 h after manufacture (Table 14), after 1 wk storage at 5°C (Table 15), or after 2 wk storage at 5°C (Table 16) among the five formulations or among the three levels of hydrolysis.

Lactose determinations were run on mix samples taken before the addition of the enzyme and after enzymatic hydrolysis was achieved to determine the exact percentage of hydrolysis. Percentages within $\pm 5\%$ of the desired percentage of hydrolysis (50 or 75%) were accepted. Table 17 shows the means of five replications. As shown in Table 18, no significant differences were detected among the five formulations but there were differences ($P<.01$) among the three levels of hydrolysis.

TABLE 10. Analysis of variance[a] of titratable acidity, 24 h after manufacture, in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .002 | |
| Rep | 4 | .010 | |
| Form | 4 | .002 | .96[NS] |
| Rep x Form | 16 | .002 | |
| Hyd | 2 | .001 | 1.04[NS] |
| Rep x Hyd | 8 | .001 | |
| Form x Hyd | 8 | .002 | 1.37[NS] |
| Rep x Form x Hyd | 32 | .001 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

TABLE 11. Analysis of variance[a] of titratable acidity, 1 wk after manufacture, in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 44 | .004 | |
| Rep | 2 | .065 | |
| Form | 4 | .002 | .66[NS] |
| Rep x Form | 8 | .002 | |
| Hyd | 2 | .0001 | .04[NS] |
| Rep x Hyd | 4 | .002 | |
| Form x Hyd | 8 | .001 | .67[NS] |
| Rep x Form x Hyd | 16 | .002 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

TABLE 12. Analysis of variance[a] of titratable acidity, 2 wk after manufacture, in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 44 | .006 | |
| Rep | 2 | .048 | |
| Form | 4 | .004 | 1.32[NS] |
| Rep x Form | 8 | .003 | |
| Hyd | 2 | .003 | .28[NS] |
| Rep x Hyd | 4 | .010 | |
| Form x Hyd | 8 | .003 | .81[NS] |
| Rep x Form x Hyd | 16 | .003 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

TABLE 13. pH values of yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| | 4% NDM[a] | | | 2% WM-34[b] 2% NDM[a] | | | 4% WM-34[b] | | | 2% MP-34[b] 2% NDM[a] | | | 4% MP-34[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 75 | 0 | 50 | 75 | 0 | 50 | 75 | 0 | 50 | 75 | 0 | 50 | 75 | Mean |
| | (pH values) | | | | | | | | | | | | | | | |
| After 24 h[c] | 4.35 | 4.40 | 4.31 | 4.45 | 4.48 | 4.40 | 4.41 | 4.45 | 4.42 | 4.36 | 4.37 | 4.39 | 4.37 | 4.41 | 4.37 | 4.40 |
| After 1 wk[d] | 4.28 | 4.30 | 4.25 | 4.26 | 4.30 | 4.29 | 4.27 | 4.33 | 4.27 | 4.25 | 4.30 | 4.25 | 4.25 | 4.28 | 4.32 | 4.28 |
| After 2 wk[d] | 4.25 | 4.27 | 4.23 | 4.21 | 4.28 | 4.23 | 4.22 | 4.22 | 4.22 | 4.22 | 4.28 | 4.25 | 4.20 | 4.20 | 4.27 | 4.24 |

[a]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[b]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

[c]Values are means of five replications.

[d]Values are means of the last three replications.

TABLE 14. Analysis of variance[a] of pH, 24 h after manufacture, in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .020 | |
| Rep | 4 | .014 | |
| Form | 4 | .023 | .40[NS] |
| Rep x Form | 16 | .057 | |
| Hyd | 2 | .013 | .87[NS] |
| Rep x Hyd | 8 | .014 | |
| Form x Hyd | 8 | .003 | .31[NS] |
| Rep x Form x Hyd | 32 | .008 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

TABLE 15. Analysis of variance[a] of pH, 1 wk after manufacture, in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DM | MS | F |
|---|---|---|---|
| Total | 44 | .008 | |
| Rep | 2 | .090 | |
| Form | 4 | .001 | .07[NS] |
| Rep x Form | 8 | .010 | |
| Hyd | 2 | .006 | 1.13[NS] |
| Rep x Hyd | 4 | .006 | |
| Form x Hyd | 8 | .002 | .48[NS] |
| Rep x Form x Hyd | 16 | .003 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

TABLE 16. Analysis of variance[a] of pH, 2 wk after manufacture, in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 44 | .010 | |
| Rep | 2 | .144 | |
| Form | 4 | .002 | .58[NS] |
| Rep x Form | 8 | .004 | |
| Hyd | 2 | .004 | .48[NS] |
| Rep x Hyd | 4 | .008 | |
| Form x Hyd | 8 | .002 | .96[NS] |
| Rep x Form x Hyd | 16 | .002 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

TABLE 17. Actual lactose hydrolysis and lactose content in finished yogurt samples manufactured from five formulations[a].

| Desired percentage of hydrolysis | 4% NDM[b] | | 2% WM-34[c] 2% NDM[b] | | 4% WM-34[c] | | 2% MP-34[c] 2% NDM[b] | | 4% MP-34[c] | | Mean | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hydrolysis | Lactose | Hydrolysis | Lactose | Hydrolysis | Lactose | Hydrolysis | Lactose | Hydrolysis | Lactose | Hydrolysis | Lactose |
| | (%) | | | | | | | | | | | |
| 0 | .00 | 5.74 | .00 | 5.69 | .00 | 5.78 | .00 | 5.89 | .00 | 5.85 | .00 | 5.79 |
| 50 | 52.08 | 3.08 | 51.25 | 3.13 | 50.05 | 3.29 | 53.11 | 3.22 | 50.93 | 3.10 | 51.48 | 3.16 |
| 75 | 75.21 | 1.51 | 76.39 | 1.47 | 74.84 | 1.63 | 73.72 | 1.72 | 73.74 | 1.74 | 74.78 | 1.61 |

[a]Values are means of five replications (tests done in duplicate).

[b]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[c]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

TABLE 18. Analysis of variance[a] of actual level of hydrolysis in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | 995.007 | |
| Rep | 4 | 1.555 | |
| Form | 4 | 3.197 | .58[NS] |
| Rep x Form | 16 | 5.521 | |
| Hyd | 2 | 36,605.316 | 2683.19** |
| Rep x Hyd | 8 | 13.643 | |
| Form x Hyd | 8 | 4.904 | .96[NS] |
| Rep x Form x Hyd | 32 | 5.130 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

**Highly significant (P<.01).

This was the expected result since the overall means for the 0, 50, and 75% hydrolyzed mixes were 0, 51.48, and 74.78% hydrolysis, respectively.

The lactose remaining after fermentation was determined in all finished yogurt samples; the means of five replications are shown in Table 17. The results of the statistical analysis are contained in Table 19. Differences ($P<.05$) were indicated among the five formulations; the lactose contents in the 4% NDM and the 2% WM-34 formulations were similar but varied significantly from 2% MP-34 formulation. The lactose in the 4% WM-34 and 4% MP-34 yogurts were also similar in content to that in the 4% NDM and 2% WM-34 yogurts. The means of the final lactose percentages from the three levels of hydrolysis within each formulation were utilized in the statistical analysis. Thus, the variability in data could be accounted for by differences in the amount of inoculation or by the variability of the age and activity of the cultures used. Differences ($P<.01$) were revealed among the levels of hydrolysis, which was expected since part of the lactose was enzymatically hydrolyzed before fermentation in the 50 and 75% hydrolyzed mixes. The final lactose contents (means of five replications) were 5.79% in the nonhydrolyzed yogurts, 3.16% in the 50% hydrolyzed yogurts, and 1.62% in the 75% hydrolyzed yogurts.

The ratio of Streptococcus thermophilus to Lactobacillis bulgaricus were determined in each finished yogurt sample. The means of five replications are summarized in Table 20. A final ratio of 1:1 but not above 1.5:1 was the aim during culturing because if an

TABLE 19. Analysis of variance[a] of final lactose percent in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | 3.045 | |
| Rep | 4 | .020 | |
| Form | 4 | .098 | 3.21* |
| Rep x Form | 16 | .031 | |
| Hyd | 2 | 13.915 | 3586.41** |
| Rep x Hyd | 8 | .031 | |
| Form x Hyd | 8 | .025 | .61[NS] |
| Rep x Form x Hyd | 32 | .041 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

*Significant (P<.05).

**Highly significant (P<.01).

[NS]Not significant.

TABLE 20. Ratios of cocci:rods in finished yogurts manufactured from five formulations[a].

| Desired percentage of hydrolysis | 4% NDM[b] | 2% WM-34[c] 2% NDM[b] | 4% WM-34[c] | 2%MP-34[c] 2% NDM[b] | 4% MP-34[c] | Mean |
|---|---|---|---|---|---|---|
| 0 | 1.18 | 1.18 | 1.26 | 1.19 | 1.08 | 1.18 |
| 50 | 1.17 | 1.11 | 1.05 | 1.16 | 1.05 | 1.11 |
| 75 | 1.11 | 1.24 | 1.07 | 1.16 | 1.11 | 1.14 |

[a]Values are means of five replications.

[b]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[c]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

imbalance had occurred and either species had become dominant, bitterness or lack of flavor would have resulted (11). As seen in Table 21, no differences (P>.05) were detected in the ratios among the five formulations; but differences (P<.05) in the bacterial ratios were revealed among the hydrolysis levels. Specifically, the means of the ratios of cocci:rods were significantly different between the nonhydrolyzed and the 50% hydrolyzed yogurts. The ratios in the 75% hydrolyzed yogurts were found to be similar to both the 0 and 50% hydrolyzed yogurts. This relationship could be attributed to the variability inherent in the daily transferring of the two bacterial species and the culturing of the yogurt. All ratio means were within the desired range of 1:1 to 1.5:1.

The calorie contents per 100 g of yogurt were computed for each yogurt sample from the equation: cal = [(% fat x 9) + {% TS - (% fat + .7%)} x 4] (37). Table 22 contains the means of five series of yogurts. As shown in Table 23, differences (P<.01) were indicated in calorie contents among the five formulations. The 4% NDM formulation varied in calories from the other four formulations while the calories in 2% WM-34 and 2% MP-34 yogurts were similar but varied from 4% NDM, 4% WM-34, and 4% MP-34 yogurts. The calories in the 4% WM-34 and 4% MP-34 yogurts were similar but varied from the calories in the 4% NDM, 2% WM-34, and 2% MP-34 yogurts. This pattern was expected since the amount of sucrose varied with the formulation; the two 50% replacement formulations contained the same amount of sucrose, as did the two 100% replacement formulations. The 4% NDM yogurts had

TABLE 21. Analysis of variance[a] of the ratio of *S. thermophilus* to *L. bulgaricus* in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .019 | |
| Rep | 4 | .015 | |
| Form | 4 | .022 | .71[NS] |
| Rep x Form | 16 | .031 | |
| Hyd | 2 | .036 | 5.87* |
| Rep x Hyd | 8 | .006 | |
| Form x Hyd | 8 | .016 | 1.06[NS] |
| Rep x Form x Hyd | 32 | .016 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

*Significant ($P<.05$).

TABLE 22. Calorie content[a] in finished yogurts manufactured from five formulations computed per 100 g of yogurt.

| Desired percentage of hydrolysis | 4% NDM[b] | 2% WM-34[c] 2% NDM[b] | 4% WM-34[c] | 2% MP-34[c] 2% NDM[b] | 4% MP-34[c] |
|---|---|---|---|---|---|
| 0 | 78.34 | 75.31 | 71.97 | 75.54 | 72.18 |
| 50 | 75.43 | 72.56 | 68.63 | 72.29 | 68.53 |
| 75 | 75.57 | 72.54 | 68.99 | 72.52 | 69.01 |

[a]Computed for each yogurt sample per 100 g from the equation (% fat x 9) + [% TS - (% fat + .7%)] x 4, and averaged from five replications.

[b]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[c]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

TABLE 23. Analysis of variance[a] of calorie content in yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | 8.758 | |
| Rep | 4 | 1.677 | |
| Form | 4 | 116.824 | 338.10** |
| Rep x Form | 16 | .0346 | |
| Hyd | 2 | 77.348 | 376.11** |
| Rep x Hyd | 8 | .206 | |
| Form x Hyd | 8 | .232 | .71[NS] |
| Rep x Form x Hyd | 32 | .324 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

**Highly significant (P<.01).

[NS]Not significant.

higher sucrose contents than the replacement formulations (Table 2).

Analysis of the data (Table 23) also detected a difference (P<.01) between the calorie contents of the nonhydrolyzed yogurts and the calories in the two hydrolyzed yogurts. Yogurts with 50 and 75% hydrolysis of lactose were similar in calorie content. This is consistent with the amount of sucrose added, since in any formulation the hydrolyzed yogurts always contained 1% less sucrose than the nonhydrolyzed yogurts (Table 2). One report (37) cited calories/100 g of 44 yogurt samples as averaging 103.21 with a range of 62.34 to 126.98 calories. The calories/100 g in this study ranged from 68.54 to 78.34 calories; all calories computed were in the lower range and well below the average reported in the previous study (37). In each of the five formulations, the hydrolyzed yogurts had significantly (P<.01) less calories than the nonhydrolyzed, which is favorable in this study. The two replacement formulations with both RMP's contained significantly (P<.01) less calories than the 4% NDM formulation since they contained less sucrose in both the nonhyrolyzed and hydrolyzed yogurts. In terms of average calorie content (the mean of 0, 50, or 75% hydrolyzed yogurts), the formulations with the highest to lowest calories were 4% NDM; 2% WM-34 and 2% MP-34; and 4% WM-34 and 4% MP-34.

All yogurt samples were found to contain acetaldehyde; the concentration of which was not quantitated. The characteristic flavor of yogurt is attributed to the by-products of lactose fermentation: lactic acid, acetaldehyde, diacetyl, and acetic acid. The proportion

of acetaldehyde among the carbonyl compounds, almost 90%, imparts a flavor to yogurt that is unlike that of any other cultured milk product (35). A relatively high concentration (.001 to .005%) of acetaldehyde will produce a well-developed yogurt flavor, whereas a product with a low concentration of acetaldehyde lacks these qualities (64).

## Dairy Panel Yogurt Evaluations

All yogurt samples were organoleptically evaluated by a panel of five Dairy Science faculty using the ADSA Swiss style yogurt score card. The score means of five replications are summarized in Table 24. Table 25 contains the results of the statistical analysis; differences (P<.01) in flavor scores were detected among the five formulations. The formulations in order from highest flavor score to lowest with each respective mean score were: 4% NDM (9.02); 2% WM-34 (8.94); 2% MP-34 (8.71); 4% WM-34 (8.36); and 4% MP-34 (8.21). Flavor scores were found to be similar among the 4% NDM, 2% WM-34, and 2% MP-34 yogurts. The 4% WM-34 and 4% MP-34 yogurts were similar in flavor to each other, but both were found to differ in flavor from the 4% NDM, 2% WM-34, and 2% MP-34 formulations. The similarity between the flavor scores of the 4% NDM, 2% WM-34, and 2% MP-34 formulations and the preference for these yogurts above the preference for the 4% WM-34 and 4% MP-34 formulations was consistent with the results cited by Gillies (24). Yogurts in that study, formulated with one half WPC and one half NDM, were either equal or superior in flavor to the control.

TABLE 24. Flavor scores, as assigned by an experienced dairy panel, on finished yogurts manufactured from five formulations[a,b].

| Desired percentage of hydrolysis | 4% NDM[c] | 2% WM-34[d] 2% NDM[c] | 4% WM-34[d] | 2% MP-34[d] 2% NDM[c] | 4% MP-34[d] | Mean |
|---|---|---|---|---|---|---|
| 0 | 9.42 | 9.14 | 8.44 | 8.92 | 8.48 | 8.88 |
| 50 | 8.94 | 8.86 | 8.28 | 8.44 | 8.00 | 8.50 |
| 75 | 8.70 | 8.83 | 8.37 | 8.76 | 8.16 | 8.56 |

[a]Based on a hedonic scale with 10 as a perfect score.

[b]Values are means of five replications.

[c]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[d]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

TABLE 25. Analysis of variance[a] of the flavor scores as assigned by an experienced dairy panel on yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .265 | |
| Rep | 4 | .162 | |
| Form | 4 | 1.866 | 9.97** |
| Rep x Form | 16 | .187 | |
| Hyd | 2 | 1.021 | 21.77** |
| Rep x Hyd | 8 | .047 | |
| Form x Hyd | 8 | .106 | .65[NS] |
| Rep x Form x Hyd | 32 | .163 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

**Highly significant (P<.01).

[NS]Not significant.

A difference ($P<.01$) in flavor scores was detected (Table 25) between the nonhydrolyzed and hydrolyzed yogurts. The nonhydrolyzed yogurts with an overall flavor score mean of 8.88 were found to be different than the 50 and 75% hydrolyzed yogurts with overall means of 8.50 and 8.56, respectively. The analysis indicated a similarity between the 50 and 75% hydrolyzed yogurt flavor scores. These results showed a significant preference for the nonhydrolyzed yogurts over the two hydrolyzed yogurts in any formulation. This is inconsistent with the results cited by Engle (17), Gyuricsek and Thompson (27), O'Leary and Woychik (53), Antila et al. (5), and Hilgendorf (30) in which hydrolyzed yogurts were preferred over nonhydrolyzed controls. They attributed the flavor preferences to the sweeter taste of the hydrolyzed yogurts imparted by the glucose and galactose liberated from hydrolyzed lactose. None of the aforementioned studies reduced the sucrose levels to allow for the additional sweetness imparted by the free glucose and galactose, as was done in this study. The most common flavor criticisms in this study were "lacks fine flavor", "low acid", "bitter", and "high acid".

The body and texture score means from five replications are presented in Table 26. The scores ranged from 4.50 to 4.90 on a scale of 1 (lowest) to 5 (highest). Differences in body and texture scores were not significant among the five formulations or among the three levels of hydrolysis. The most frequent body and texture criticisms were "weak" and "free whey". Table 27 contains the results of the statistical analysis of body and texture scores.

TABLE 26. Body and texture scores, as assigned by an experienced dairy panel, on finished yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis[a,b] of lactose.

| Desired percentage of hydrolysis | 4% NDM[c] | 2% WM-34[d] 2% NDM[c] | 4% WM-34[d] | 2% MP-34[d] 2% NDM[c] | 4% MP-34[d] | Mean |
|---|---|---|---|---|---|---|
| 0 | 4.85 | 4.90 | 4.65 | 4.63 | 4.63 | 4.73 |
| 50 | 4.84 | 4.63 | 4.45 | 4.52 | 4.54 | 4.60 |
| 75 | 4.77 | 4.60 | 4.67 | 4.70 | 4.50 | 4.65 |

[a]Based on a hedonic scale with five as a perfect score.

[b]Values are means of five replications.

[c]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[d]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

TABLE 27. Analysis of variance[a] of body and texture scores as assigned by an experienced dairy panel on yogurts manufactured from five formulations with 0, 50, or 75% lactose hydrolysis.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .103 | |
| Rep | 4 | .334 | |
| Form | 4 | .172 | .94[NS] |
| Rep x Form | 16 | .184 | |
| Hyd | 2 | .121 | 3.68[NS] |
| Rep x Hyd | 8 | .033 | |
| Form x Hyd | 8 | .040 | .72[NS] |
| Rep x Form x Hyd | 32 | .056 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

[NS]Not significant.

Consumer Panel Flavor Evaluations

All yogurt samples were also organoleptically evaluated by a consumer panel with not less than 25 persons per taste test. Each taste test was performed by different persons who were recruited randomly. The tasters were not screened as to yogurt consumption and preference. The means of the flavor scores from five series of yogurts are tabulated in Table 28. The yogurts were judged on a hedonic scale of 1 (extremely disliked) to 9 (extremely liked). Results ranged from 4.21 to 6.11. These low scores were largely attributed to the fact that the yogurt was plain and recruited volunteers could have scored the yogurts lower because of their preference for flavored yogurt. In a survey of 400 households (36), 161 indicated occasional or frequent yogurt consumption; of the 161 households, 74% preferred fruit-flavored yogurt while only 21% like and ate plain yogurt.

Table 29 contains the results of the statistical analysis performed on the data from the consumer panel. Differences (P<.01) were found in the flavor scores among the five formulations. The consumer flavor scores indicated a preference for the yogurt formulations in the same order as the Dairy Science panel: 4% NDM (score of 5.91); 2% WM-34 (5.60); 2% MP-34 (5.12); 4% WM-34 (4.62); and 4% MP-34 (4.54). Thus, the 50% replacement levels of NDM by RMP's were preferred over the 100% replacement formulations, which is consistent with the results cited by Gillies (24). The analysis indicated a similarity in scores between 4% NDM and 2% WM-34, which were both different than the scores for 4% WM-34 and 4% MP-34. A similarity in scores was noted between

TABLE 28. Flavor scores, as assigned by a consumer survey, on finished yogurts manufactured from five formulations[a,b].

| Desired percentage of hydrolysis | 4% NDM[c] | 2% WM-34[d] 2% NDM[c] | 4% WM-34[d] | 2% MP-34[d] 2% NDM[c] | 4% MP-34[d] | Mean |
|---|---|---|---|---|---|---|
| 0 | 6.11 | 5.68 | 4.94 | 5.50 | 4.80 | 5.41 |
| 50 | 5.72 | 5.65 | 4.52 | 4.84 | 4.21 | 4.99 |
| 75 | 5.92 | 5.46 | 4.38 | 5.04 | 4.60 | 5.08 |

[a]Based on a hedonic scale with nine as a perfect score.

[b]Values are means of five replications.

[c]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[d]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

TABLE 29. Analysis of variance[a] of the flavor scores as assigned by a consumer survey on yogurts manufactured from five formulations with 0, 50, or 75% hydrolysis of lactose.

| Source | DF | MS | F |
|---|---|---|---|
| Total | 74 | .814 | |
| Rep | 4 | 3.545 | |
| Form | 4 | 5.428 | 6.91** |
| Rep x Form | 16 | .785 | |
| Hyd | 2 | 1.199 | 4.18[NS] |
| Rep x Hyd | 8 | .287 | |
| Form x Hyd | 8 | .127 | .67[NS] |
| Rep x Form x Hyd | 32 | .189 | |

[a]Analysis of variance using 5 x 3 factorial design with five replicates.

**Highly significant (P<.01).

[NS]Not significant.

2% WM-34 and 2% MP-34; and between 4% WM-34, 2% MP-34, and 4% MP-34. Flavor scores among levels of hydrolysis were not different (P>.05); however, nonhydrolyzed yogurts (score of 5.40) were preferred over the 50% (4.99) and 75% (5.08) hydrolyzed yogurts.

Costs of the Yogurt Mixes

Costs of the fifteen different yogurt mixes based on the experimental 1.89 liter sized batches are shown in Table 30. Prices of the mixes were calculated using March, 1982 prices of 2% lowfat milk, sucrose, NDM, WM-34, MP-34, and lactose hydrolysis enzyme (MAXILACT® LX5000). Since each RMP was cheaper than NDM, both the 50 and 100% replacement formulations for WM-34 and MP-34 were less expensive than the 4% NDM formulation. Unfortunately, MAXILACT® LX5000 is $150/kg even if purchased in volume (2500 kg). Thus, the cost of the enzyme used per 1.89 liters of milk was more expensive than the cost of the sugar not added to the hydrolyzed batches. Hence, as seen in Table 30, the hydrolyzed mixes were more expensive than the corresponding nonhydrolyzed mixes in each formulation.

A less expensive enzyme, MAXILACT® L2000, is available for food-grade use. Dosages would have to be increased 2.5 times the dose for MAXILACT® LX5000 for the same holding period. At a cost of $35/kg the cost per 1.89 liters would be $.05 compared to $.09 using MAXILACT® LX5000. Costs would be $.04 less per 1.89 liters but still $.02 more than the nonhydrolyzed mixes. It is felt, however, that with calorie and diet conscious consumers, the slight cost premium would be readily

TABLE 30. Costs ($) of yogurt mixes[a].

| Desired percentage of hydrolysis | 4% NDM[b] | 2% WM-34[c] 2% NDM[b] | 4% WM-34[c] | 2% MP-34[c] 2% NDM[b] | 4% MP-34[c] |
|---|---|---|---|---|---|
| 0 | 1.39 | 1.28 | 1.18 | 1.29 | 1.19 |
| 50 | 1.45 | 1.35 | 1.24 | 1.36 | 1.25 |
| 75 | 1.45 | 1.35 | 1.24 | 1.36 | 1.25 |

[a]Costs are based on 1.89 liter sized mix; cost includes March prices of 2% lowfat milk, sucrose, NDM, WM-34, MP-34, and enzyme.

[b]Nonfat dry milk, Land O'Lakes, Inc., Minneapolis, MN.

[c]Reconstructed milk products, Dairyland Products, Inc., Savage, MN.

accepted for the reduced calorie product.

## SUMMARY AND CONCLUSIONS

Yogurts were formulated from 2% lowfat milk with 4% added nonfat dry milk or reconstituted milk products (RMP), with 1 to 4% sucrose depending upon the formulation and whether lactose was hydrolyzed, and with .5% gelatin stabilizer. A formula with 4% added (by weight) NDM was chosen as representative of commercial yogurts. Two RMP's formulated to match the functional, chemical, and nutritional properties of NDM were chosen as economical replacements for NDM at the 50 and 100% levels of replacement in experimental formulae. It was intended to reduce the ingredient costs of yogurt by these replacements without impairing flavor.

The lactose in some of the yogurt mixes was partially hydrolyzed enzymatically at two levels (50 and 75%) in order to provide additional sweetness via the end products of lactose hydrolysis. This would allow the added sucrose level to be reduced to maintain the original degree of sweetness. It was thought the cost of manufacturing could be reduced by using less sucrose; the calorie content lowered to attract diet-conscious consumers; and a product with lowered amounts of lactose provided for lactose sensitive persons without impairing the flavor of the yogurts.

There were five different formulations which contained 4% NDM, 2% WM-34 plus 2% NDM, 4% WM-34, 2% MP-34 plus 2% NDM, or 4% MP-34, and three hydrolysis levels (0, 50, or 75%) for a total of fifteen formulations. Five series of yogurts were made by each formula; analyzed for composition; evaluated by a panel of Dairy Science faculty for

flavor, body, and texture; and evaluated by a consumer panel for preference.

The mean values of the compositional analyses of all the yogurt mixes (averaging all formulations and hydrolysis levels) were 4.94% protein, 1.92% fat, 16.47% total solids, 1.02% ash, and 6.98% lactose. While the protein, fat, ash, and lactose should have been constant in all the mixes, the total solids content varied depending upon the formulations due to the varying sucrose levels.

A panel of Dairy Science faculty detected flavor differences ($P<.01$) in the yogurts with 100% replacement of NDM by the RMP's compared to yogurts with no replacement of NDM; however, all flavor scores were at least 8.0 or above on a 10 point scale. No differences ($P>.05$) were detected between the yogurts with no replacement of NDM and the yogurts with 50% of the NDM replaced by RMP's. No significant differences were detected in body and texture among yogurt samples. Differences ($P<.01$) in flavor between the hydrolyzed yogurts and the nonhydrolyzed yogurts were detected. The nonhydrolyzed yogurts were preferred although the overall means of the hydrolyzed yogurts (8.50 for the 50% and 8.56 for the 75% hydrolyzed yogurts) indicated good acceptability of these yogurts, too.

A consumer panel detected flavor differences ($P<.01$) in the 100% replacement formulations of NDM by WM-34 and MP-34 and in one formulation (MP-34) of 50% replacement of NDM. Yogurts made with 2% WM-34 were indistinguishable in taste from the 4% NDM yogurts. The consumer panel detected flavor differences among the nonhydrolyzed

and hydrolyzed yogurts but these differences were not statistically significant (P>.05).

Calories were significantly reduced (P<.01) among the formulations in which the sucrose had been reduced in connection with substitution of RMP's for NDM and/or hydrolysis of lactose in the yogurt mixes. It can be concluded that a lower calorie yogurt can be manufactured but at a slight expense of flavor if enzymatic hydrolysis is used in conjuction with sucrose reductions to lower the calories. While the consumer panel did not detect significant flavor differences between hydrolyzed and nonhydrolyzed yogurts, the Dairy Science panel did detect highly significant flavor differences in favor of the nonhydrolyzed yogurts.

Reconstructed milk products or similar whey-blend products manufactured to the same functional and nutritional properties as NDM may be used as substitutes for NDM at the 50% replacement level in yogurt without loss of organoleptic properties. Complete substitution of RMP's for NDM is questionable. These types of products are allowed by the Federal Standards of Identity (18) as optional ingredients in the manufacture of yogurt but only to the extent that the consumer will accept the product. With further development in whey processing technology, perhaps some other whey-containing products will be manufactured that cannot be detected at the 100% replacement level. It was found that the use of whey protein products can lower the cost of manufacturing yogurt, but the cost savings depends upon the use level and the cost of the particular product.

Enzymatic hydrolysis of lactose using MAXILACT® LX5000 increased the cost of manufacture since the amount of sucrose omitted in this study was less expensive at this time than enzyme preparation used. A less expensive and less purified enzyme is available but is still slightly more expensive than the sucrose omitted. Use of these enzyme preparations might become economically feasible if sucrose rises in price or enzyme costs are reduced. The economics of hydrolyzing lactose with immobilized enzyme technology may prove to be favorable in the future, but it was not undertaken in this study.

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