

CHAPTER I

INTRODUCTION

The food industry has become more consumer driven (Kinsey and Senauer, 1996). The system is being designed to respond more quickly to the consumer. Consumer preference for reformulated products with specific food characteristics is growing. Food technologists have responded to these demands by providing a variety of new partially processed, high quality, extended shelf life foods (Rhodes, 1991). Successful food companies are organized to deliver such foods to the consumer and are targeting more products at particular market segments (Senauer, 1990).

American demographics are changing to include a greater ethnic diversity, a larger segment of older adults, and a greater number of women in the work force (Kinsey and Senauer, 1996). By the year 2025, the growing number of ethnic groups (Hispanics, Asians, and African- Americans) will contribute to over 38% of the American population. The population of elderly individuals, 65 years and older, will increase from 13% in today's population to over 18% (62 million people) by the year 2025. The increased number of women in the labor force is affecting changes in the food industry. In 1970, only 43% of women were in the work force compared to 59% in 1994.

The dynamic changes in the U.S. population demographics have caused many lifestyle trends that influence consumer attitudes toward food. A 1992 U.S. food marketing survey showed that consumers ranked convenience, storability, taste, nutrition, price, and product safety as important factors when making food selections. Convenience and storability were very important to three-fourths of the people surveyed (Food Marketing Institute, 1992). Convenience foods save consumers time by reducing the time needed to prepare a food prior to consuming the product (Kinsey and Senauer, 1996). Consumers are usually willing to pay more for these products due to their increased convenience. Consumers expect these foods to possess the taste and the quality of home-made entrees. A 1992 U.S. food marketing survey showed that 96% of consumers ranked

taste as another important factor in their food decisions (Food Marketing Institute, 1992).

Nutrition is also very important to the consumer. In 1991 and 1992, two national consumer surveys were reported that provided some insight on consumers nutrition concerns (Christian and Greger, 1994). Fat and cholesterol contents of food were primary nutrition concerns for U.S. consumers (American Dietetic Association, 1991; Food Marketing Institute; 1992) . Consumers have reduced their consumption of eggs because of the relationship between dietary cholesterol and heart disease (Paraskevopoulou and Kisseoglou, 1997; Warren et al., 1991). There has also been a reduction in the consumption of whole milk (IDFA, 1996). From 1974 to 1996, the per capita sale of fluid whole milk decreased from 172.3 pounds to 72 pounds, respectively. However, the per capita sales of lowfat and skim milk from 1974 to 1996 increased from 59.7 lbs to 116 lbs, respectively (IDFA, 1996). The decline of whole milk consumption is attributed to health concerns related to saturated fat, cholesterol, and calories (Bunch, 1985; Berner and Lofgren, 1991). In addition to saturated fat and cholesterol in milk, the presence of lactose is also influential in reduction of milk consumption.

Approximately 50 million Americans, many with ethnic backgrounds, have some difficulty digesting milk and milk products due to the presence of lactose (Reiter, 1991). These individuals are unable to breakdown the lactose in milk because of the absence or deficiency of the intestinal enzyme *B-* galactosidase (Houts, 1988). Therefore, they may avoid milk and milk products to avoid symptoms such as abdominal distention, cramping, gas, and/or diarrhea due to the presence of lactose in these products. In response to this problem, biotechnology has made it possible to economically produce enzymes that break down the lactose into the monosaccharides, glucose and galactose which are more digestible by the body.

Consumers base many of their food purchasing decisions on the nutritional profile of products and look to the food industry to manipulate the nutrients in foods to help improve dietary choices (Frazao and Allshouse, 1996). Although dairy foods contain fat,

cholesterol, and lactose, all nutrients of concern to many consumers, dairy foods are nutritious and healthy foods that offer nutritional benefits to consumers (Berner and Lofgren, 1991). The dairy industry must handle consumer concerns by addressing nutritional concerns as well as any other concerns. Consumers want nutritious foods that taste and perform as well as the traditional products (Frazao and Allshouse, 1996). Until recently, nutritionally improved versions of products failed to meet consumer taste preferences. The food industry has responded to consumer concerns about nutritional profile of foods by reformulating many traditional products.

A study of U.S. supermarkets, conducted between 1989 and 1993, showed the sale of nutritionally improved foods grew at a faster pace than the sale of the regular versions of the products (Frazao and Allshouse, 1996). Nutritionally improved foods generally cost more than the regular versions, but price is of less importance to many consumers than nutrition, taste, and convenience. In the first quarter of 1995, nearly 1000 claims were made on improved nutritional profiles of products such as “reduced fat”, low sodium, and low cholesterol.

The volume share of nutritionally improved milks increased from 55% in 1989 to 67% in 1993 (Frazao and Allshouse, 1996). However, milk with a lower fat content cost less than whole milk so the profit from increased sales was reduced. The category of nutritionally improved milks included milks with lower fat and cholesterol. Individuals that switch from whole milk to skim milk reduce their intake of fat and saturated fat. Refrigerated puddings, a relatively new product category for the dairy industry, showed impressive growth of over 23,000 percent between 1989 to 1993. The nutritionally improved version costs more than traditional products. This category of products is relatively small compared to other dairy product categories but demonstrates that development of new dairy products offering nothing but positive attributes to the consumer could advance growth opportunities in the dairy foods market. Meeting consumer expectations results in increased sales of value-added dairy products that

generally have higher retail prices than traditional products.

The goal of this project was to develop a nutritionally enhanced fluid milk and egg mixture that can be used in a variety of applications ranging from desserts to main entrees. The convenience characteristics of this product in combination with the nutrition enhancement would result in a value added dairy product. The products made with this milk and egg mixture could be targeted to food service operations.

The first objective of this study was to formulate a nutritionally enhanced product that retained the functionality and quality of the ingredients under appropriate thermal processing conditions adequate for guaranteeing food safety.

The second part of this study was an extended shelf life study in which the objective was to monitor the mixture over a seven week period noting any quality changes that may occur in the control and nutritionally modified egg and milk mixture over time.

CHAPTER II

REVIEW OF LITERATURE

A. Nutrition Concerns of the American Population

The average American diet contains too much fat especially saturated fat, as well as cholesterol, sodium, and too many calories for the level of physical activity (Senauer, 1990). Almost two-thirds of the two million deaths in the U.S. each year are connected to heart disease, cancer, and strokes. Studies have provided evidence that these causes of death are affected by diet as well as other lifestyle factors. In dairy foods, consumers state that fat, cholesterol, and calorie intake are the most important components of concern (Berner and Lofgren, 1991). From 1983 to 1987, concern about fat and cholesterol increased among consumers. The average amount of calories from fat has decreased from 40% in 1977 to 37% in 1990 (Senauer, 1990). In dairy foods, lactose is also another component of concern for some individuals. Lactose, a milk sugar found in dairy products, makes it difficult for some individuals to digest dairy products because the individual experiences some type of gastrointestinal discomfort (Solomons, 1986).

Fat and Cholesterol. A Surgeon General's report on Nutrition and Health lists heart disease, cancers, and strokes as the leading cause of death in the U.S. with diet being associated with the incidence of these diseases (USDA, 1988). Thus, the report recommended a reduced consumption of dietary saturated fat and cholesterol.

Studies have shown that high intakes of total fat, saturated fat, and cholesterol are associated with elevated low-density lipoproteins (LDL) (McNamara, 1992). In recent years blood lipoproteins have been associated with coronary heart disease risk. Low-density lipoproteins are the major cholesterol carrying lipoproteins in the blood. Low-density lipoproteins at elevated levels increase the risk of coronary heart disease. In 1989, coronary heart disease claimed over 700,000 lives with an estimated cost of \$50 to \$100 billion a year for medical treatment and lost wages (Miller et al., 1995).

The American Heart Association suggests that fat be limited to 30% of total

calories with an equal portion of calories coming from saturated, monounsaturated, and polyunsaturated fats (Watkins, 1995). Other guidelines issued by federal government agencies and health organizations recommend that Americans consume a diet with no more than 30% of the energy from fat, no more than 10% of energy from saturated fat, and no more than 300 mg of cholesterol a day (Miller et al., 1995). To meet these guidelines lower fat dairy products are recommended. In 1990, with the exclusion of butter, dairy products contributed 12% of total fat, 20% of saturated fat, and 15% of cholesterol in the U.S. food supply (Miller et al., 1995).

Milk fat has a fatty acid profile consisting of 62% saturated fats, 30% monounsaturated fats, and 4% polyunsaturated fats. Saturated fatty acids such as palmitic (C16:0), myristic (C14:0), and lauric (C12:0) raise blood cholesterol levels, whereas stearic (C18:0) and medium chain fatty acids have little effect on raising blood cholesterol levels. The American diet receives only 5 to 7% of its energy from polyunsaturated fats which is below the recommended 10% of total energy from polyunsaturated fats.

Cholesterol is a sterol found in eggs, milk, and foods of animal origin (Potter, 1986). These foods are believed to increase the blood cholesterol. The increase in blood cholesterol levels is due to an increase in low density lipoprotein of cholesterol (Miller et al., 1995). Cholesterol is a contributing factor of atherosclerosis a major cardiovascular disease.

In response to consumer concern about the levels of dietary fat and cholesterol in their diet the dairy industry has been developing new products that are lower in fat and cholesterol (Berner and Lofgren, 1991). In 1992, new and healthier milk products were introduced to the consumer with such health claims as low fat. Farmland Dairies, Wallington, N.J. and Oakhurst Dairy, Portland, Maine introduced cholesterol reduced milks (Friedman and Dornblaser, 1992).

Lactose. Approximately 50 million Americans have some difficulty digesting

milk and milk products because of the lactose content (Reiter, 1991). Incomplete digestion of lactose due to low levels of the lactase enzyme in the human intestinal tract may cause a condition known as lactose intolerance (McBean, 1994). Lactose intolerance is the symptomatic response to the consumption and malabsorption of lactose from any cause. The unpleasant symptoms cause many persons to stop consuming milk and other dairy products (Shah, 1993; Newcomer and McGill, 1984). Some symptoms that are experienced by lactose intolerant individuals are nausea, gas in the abdomen and intestines, abdominal cramping and distention, flatulence, and/or diarrhea (Solomons, 1986). A demographic profile on the estimated numbers of North American adults who are lactose intolerant has been developed by the National Dairy Council (Reiter, 1991). Lactose intolerance is found in approximately 94% of Asians, 79% of Native Americans, 75% of African Americans, 70% of Jews, 51% of Hispanics, and 21% of Caucasians. Lactose intolerance incidence increases with age from decreased lactase activity (Fox, 1985).

Nutrition Considerations for Lactose Intolerant. Researchers are concerned with the nutritional implications of being lactose intolerant (McBean, 1994). It is generally believed that elimination of dairy products is unnecessary and nutritionally unwise in the lactose intolerant population. Studies show that reduced calcium intake leading to bone fragility seems to be more common in lactose maldigesters (Miller et al., 1995). However, more studies need to be done to determine whether increased bone fragility in lactose maldigesters is due to the reduced intake of milk products or impaired calcium absorption.

In the U.S., milk and milk products have been major foods in the U.S. diet (National Research Council, 1988). In 1990, 75% of the calcium in the U.S. food supply came from milk and milk products (McBean, 1994). Milk and milk products also provide nutrients such as protein, riboflavin, vitamin B12, vitamin D, and vitamin A. Thus, lactose-reduced dairy products are recommended for the lactose intolerant consumer as a

way of increasing their daily consumption of dairy products (Houts, 1988). However, lactose aids in the absorption of calcium by the body (Heller, 1988).

B. Overview of Composition, Nutritional Value, and Functionality of Eggs and Milk

1. Eggs

Composition. There are four distinct parts to a whole egg: shell, shell membrane, egg white, and yolk (Hui, 1992). Focus will be placed on the egg white and egg yolk as they contribute to nutrition and functionality in foods. The edible proportion of egg white to egg yolk in a large raw egg (50 g) is 67% egg white to 33% egg yolk (American Egg Board (AEB), 1989).

The egg white (albumen) is made up of four layers: outer thin albumen, thick albumen, inner thin albumen, and chalaziferous layer (Powrie and Nakai, 1985). Water constitutes 87 - 89 % of the albumen. Protein, the major component of albumen solids, is 9.7- 10.6%. The egg white proteins include ovalbumin, conalbumin, ovomucin, ovoglobulins, ovomucid, and lysozyme (Hui, 1992). The ovalbumin is the predominant protein (Chan et al., 1995). Egg white proteins contain all of the essential amino acids in a well balanced proportion for human health (Hui, 1992). Albumen contains negligible amounts of lipid, 0.3% when compared to egg yolk (Powrie and Nakai, 1985). Albumen also contains carbohydrates in the free form and in combination with protein at levels of about 1%. Ninety-eight percent of the free carbohydrates in albumen is glucose.

The yolk has a total solids content of about 50%, but the solids content decreases during egg storage due to the migration of water from the albumen to the yolk (Chan et al., 1995). Lipids and proteins are the major constituents of the egg yolk solids. The lipid content of egg yolk varies between 32 - 36% depending on the strain of hen and the diet (Powrie and Nakai, 1985). The yolk lipid consists of 65.5% triglycerides, 28.3% phospholipids, and 5.2% cholesterol (Privett et al., 1962). A large portion of the lipid in the yolk is bound to the protein in the form of lipoprotein complexes (Conte, Jr. et al., 1992). The cholesterol content on average for one large egg is about 213 mg (AEB,

1997).

The protein content of egg yolk has a range of 15.7 - 16.6 % (Powrie et al., 1985). Egg yolk consists of yolk spheres, granules, low density lipoproteins, and myelin figures (McWilliams, 1993). When centrifuged, the yolk can be separated into a plasma phase and a granule phase containing these particles (Chan et al., 1995). Egg yolk's plasma contains livetin, a prominent globular protein, and low density proteins (McWilliams, 1993). The low density lipoproteins in the plasma consists largely of lipids with the protein level being just over 10%.

The egg yolk granules contain three types of protein lipovitellins, phosvitin, and low density lipoproteins. The lipovitellin, a high density lipoprotein, consists largely of proteins and the lipid level is only about 20%. Lipovitellin is the most abundant granular protein accounting for 70% of the granule. In addition to lipovitellin, phosvitin accounts for 16% of the granule and low density lipoproteins account for 12% of the granule. Phosvitin has the ability to bind ferric ions and incorporate it into the yolk.

Nutritional Value. Eggs are a well balanced source of nutrients for humans (AEB, 1989). Eggs are an important source of protein, essential unsaturated fatty acids, monounsaturated fatty acids, iron, phosphorus, trace minerals, fat soluble vitamins A, D, E, and K and water soluble B vitamins (Watkins, 1995). The protein in eggs is of such high quality it is often used as a standard of protein measurement (AEB, 1997). Eggs provide essential sources of linoleic acid and proportions of saturated, monounsaturated, and polyunsaturated fats in ratios suggested by the American Heart Association (Watkins, 1995). The egg yolk makes up slightly over one third of the egg. However, it yields 78% of the calories and provides most of the fat, calcium, phosphorus, iron, zinc, vitamins B₆, B₁₂, and A, folic acid, pantothenic acid, and thiamine, and almost half the protein and riboflavin of egg. However, egg albumen contains more than half of the total protein and riboflavin since there is proportionally almost twice as much albumen than yolk.

Eggs offer an excellent source of nutrients for young children and teenagers

during rapid growth. Eggs are valuable foods for patients recovering from illness because of high digestibility and high nutrient content. Eggs are usually included in the first low calorie or soft diets of these patients. Eggs are very beneficial and acceptable in diets of the elderly who have lower caloric needs but have greater difficulty digesting and absorbing nutrients.

Adequate nutrient intake is critical for the elderly since age related changes occur in the gastrointestinal tract and the diet can profoundly influence their immune system (Symposium on Nutrition and Aging, 1992).

There are some components of eggs that have negative dietary implications. There has been a decline in egg consumption due to recommendations by health professionals and some nutritionists to reduce cholesterol and saturated fat content in the diet (Paraskevopoulou and Kiosseoglou, 1997). Saturated fat tends to elevate blood cholesterol levels. The yolk contains cholesterol which is a concern for people who are limiting their cholesterol intake (McWilliams, 1993).

Processing Methods of Removing Cholesterol and Fat from Eggs. There are several different processing methods used to remove or reduce the cholesterol and lipids from egg yolks (Froning, 1995). These methods include organic solvent extraction, supercritical carbon dioxide extraction, and the use of edible oils as an extraction method.

Organic solvents have been used to extract cholesterol and lipids from egg yolks. Warren et al. (1988) reported that mixtures of hexane: isopropanol and chloroform: methanol were effective in reducing the cholesterol in egg yolks. However, organic solvents are not selective for cholesterol and also extract phospholipids which are important for functionality and organoleptic qualities. In addition, the solvents denature proteins important in functionality of a product. Residue and environmental concerns on disposal and handling of the organic solvents in a food industry plant is also a problem (Froning, 1995).

Supercritical carbon dioxide extraction offers a means of selectively extracting lipid components from food (Froning, 1995). This method of extraction has several advantages over solvent extraction. Carbon dioxide is readily available and nontoxic, unlike organic solvents (Warren et al., 1991). Also, the process can occur at low temperatures minimizing heat damage to the product. A study by Froning et al. (1990) showed that the extraction did not remove substantial quantities of phospholipids which are important for the functionality of egg yolk. The NutraSweet Company, Deerfield, Illinois used the supercritical carbon dioxide extraction in their development of a low-fat, low-cholesterol egg yolk product (Kevin, 1995). The egg yolk was marketed in 1993 but has since been discontinued.

Edible oils containing monoglycerides to remove cholesterol from liquid egg and liquid egg yolk (US patent 5091203, Conte, Jr. et al., 1992). The edible oil is emulsified with the eggs in an aqueous solution. The mixture is heated and stirred (low shear) to ensure the oil and aqueous phases come into intimate contact with each other. The stirring is continued until there is an exchange of cholesterol from the aqueous phase to the lipid phase. Finally, the cholesterol and fat reduced aqueous egg phase is separated from the cholesterol enriched edible oil phase by centrifugation or gravity separation.

Functional Properties of Eggs. Eggs are very important to the food industry because they offer many desirable attributes as a food ingredient (AEB, 1997). Functional properties contributed by eggs include coagulation, emulsification, foaming, coloring, flavoring, and control of crystallization. Providing such functional properties as these make it difficult to replace eggs in food products (Penfield and Campbell, 1990).

In custard products the major function of eggs is coagulation of the proteins to form a gel. Coagulation is used to describe the change from a fluid to a solid state caused by the denaturation and aggregation of both egg yolk and egg white proteins (Yang and Baldwin, 1995). Applying heat, mechanical agitation, salts, acids, and alkalines to proteins may cause coagulation. The formation of coagulum is often thermally

irreversible (Shimada and Matsushita, 1981). The coagulum's three dimensional network of proteins is formed by intermolecular hydrophobic bonds, hydrogen bonds, and disulfide bonds. The first step in heat coagulation involves the formation of disulfide bonds and the exposure of hydrophobic groups (Shimada and Matsushita, 1980). The denatured proteins form soluble aggregations and further heating causes the egg protein network to form by inter-molecular sulfhydryl-disulfide exchanges (Mori et al, 1986; Shimada and Matsushita, 1980).

Egg white conalbumin is more heat sensitive than the ovalbumin and has a tendency to undergo protein denaturation more rapidly (Johnson and Zabik, 1981). All of the egg yolk proteins are subject to heat coagulation except phosvitin and some livetins (Yang and Baldwin, 1995). The aggregation of the denatured proteins increases the viscosity of a mixture and, when the protein concentration is adequate, the mixture may coagulate into a solid (McWilliams, 1993). The low density lipoproteins in the egg yolk contribute to forming stable gels under a pH range of 4-9 (Yang and Baldwin, 1995).

There are several factors which affect or influence the coagulation of egg proteins: temperature, dilutions, salts, sugar, acid, and alkali. Egg whites begin to coagulate at 62°C while egg yolks begin to coagulate at 65°C. Excess heat, from high temperatures or excess exposure time, causes a very firm gel but also causes syneresis, an undesirable characteristic. Diluting egg proteins raises the coagulation temperature and decreases the firmness of the coagulum (Beveridge et al., 1980). Salt addition usually promotes coagulation if added in the proper amount. Substituting water for milk in custard mix reduced salt concentration but a gel did not form (Yang and Baldwin, 1995). However adding salt to the water- egg mix caused coagulation of the gel.

Sugar affects coagulation by raising the coagulation temperature (Wolfe and Zabik, 1968). A study by Dixon and Cotterill (1981) monitoring the electrophoretic and chromatographic changes in egg yolk proteins due to heat showed that sugared or salted egg yolk and whole egg can be pasteurized at higher temperatures, approximately 68 °C,

than when sugar or salt were not added. Wang et al. (1973) showed that addition of sucrose to custard caused a weaker gel structure. However, when the milk and egg solids were held constant, sucrose did not affect the gel strength. The effects that acids and alkali have on coagulation depends on the pH and its relation to the isoelectric point of the proteins. When proteins are heated at their isoelectric point, a gel with large pores, weak gel formation, and minimal water binding occur. However at a high pH a fine uniform gel with high gel strength and improved water binding occurs (Yang and Baldwin, 1995).

Processed Eggs. Dried eggs are manufactured by removing or evaporating water from the eggs to a level to stop microorganism growth and slow down chemical reaction rates (Bergquist, 1995). Advantages to using dried egg products are: require less space than shell or liquid eggs; have lower transportation costs; are less susceptible to bacterial growth when stored properly; provide good uniformity; and may be used in the development of new convenience foods. Dried egg products are used in bakery foods, mixes, mayonnaise and salad dressings, confections, ice cream, and pastas. Dried egg products must meet specific chemical, physical, functional, and microbiological specifications. There are many types of dried egg products: spray-dried egg white (whipping and nonwhipping type), pan-dried egg white, stabilized (glucose-free) egg yolk, standard egg yolk, standard whole egg, stabilized whole egg, etc.

Spray drying is one method of processing dried eggs. The liquid egg portion is atomized into a stream of hot air and moisture is removed very rapidly until the solid portion remains with a minimal moisture content. Every attempt is made to preserve the native characteristics of the egg. The functional properties of these dried ingredients may be altered due to the drying process. During spray drying, pumping and atomization of the egg exposes the proteins to shear force which disrupts the native state of the proteins. Under pressures of 5000 psi, the viscosity and functionality of egg white is not changed. Eggs are affected more by the drying process if the glucose is not removed (Sebring,

1995). The presence of glucose in the eggs can cause egg products to become less soluble and cause formation of off-colors and flavors from reaction between glucose and proteins during subsequent storage. Thus, the removal of glucose improves the stability of the eggs. Glucose can be removed by yeast, bacterial, or enzymatic oxidation before drying of egg whites and yolks (Forsythe, 1953).

The nutrient content of dried eggs is not lost under proper processing conditions (Bergquist, 1995). Cotterill et al. (1978) found that the nutrient composition of spray-dried eggs was essentially the same as liquid counterparts. Nutrients such as vitamin A and B, thiamine, riboflavin, pantothenic acid, and nicotinic acid were similar to the fresh egg product.

2. Milk

Composition. The major components of milk are water, milkfat, protein, lactose, and phosphorus and minerals (Eskin, 1990). The most variable component of milk is fat which may vary from 3.0 to 3.8 percent in fresh raw milk (Penfield and Campbell, 1990). Milk lipids are present in fat globules ranging from 0.1 to 20 μ m in diameter (Eskin, 1990). The fat globules are separated from the aqueous milk serum by a milk fat globule membrane (MFGM). The MFGM layer contains triacylglycerols, free fatty acids, monoglycerides, phospholipids, traces of cholesterol esters, enzymes, proteins, and glycoproteins. The MFGM layer influences the stability of the fat layer in milk and the changes that occur during processing. The triacylglycerols make up about 97-98 % of the milk fat (Swaisgood, 1985). The fatty acids of the triacylglycerols vary depending on the breed of cow and management practices.

The milk proteins are composed of casein and serum proteins (Eskin, 1990). Casein comprises about 80% of the protein in bovine milk. Casein and calcium phosphate form a highly hydrated spherical complex called a micelle (Swaisgood, 1985). Casein micelles are macromolecular aggregates ranging in size from 30 to 300 nm. The casein micelles are responsible for the whitish opaque color in milk. The casein micelle

not only contains protein, it contains small ions such as calcium, phosphate, magnesium, and citrate and is referred to as colloidal calcium phosphate. The stability of micelles impart a great influence on the processing properties of milk. The interaction of the calcium, phosphate, magnesium, and citrate with the proteins contributes to the stability of dairy products. The major casein fractions are α_{s_1} -, α_{s_2} -, k-, β -, and γ - caseins (Eskin, 1990). The difference between the casein fractions is how they react toward calcium ions. β - casein is the most temperature dependent and α_{s_2} casein is less sensitive to temperature and more sensitive to ionic strength.

Lactose is a disaccharide composed of glucose and galactose linked by an α -1,4 glycosidic bond (Eskin, 1990). It is the major carbohydrate of milk accounting for 50% of the solids in skim milk (Swaisgood, 1985). One cup of milk contains anywhere from 11 to 12 grams of lactose (McWilliams, 1993). There is about 6.75 % lactose present in human milk and 4.8 % present in cow's milk. Lactose occurs in two forms α - lactose and β -lactose; the β form is more soluble. The sweetening power of lactose is only about one-fifth that of sucrose (Swaisgood, 1985).

Production of Lactose Reduced Dairy Products. There are certain requirements for lactase to be used in milk hydrolysis (Repelius, 1983). The enzyme should be from a safe source; it should be active at the natural pH of milk (pH 6.7-6.8); it should be free of any other materials that may alter the milk causing bacterial spoilage, protein denaturation, and/or browning as a result of hydrolysis; and it should also be active at low temperatures (6-8 °C).

Major commercial sources of lactase for industrial applications are obtained from the yeast (*Kluyveromyces fragilis* and *Kluyveromyces lactis*) and the fungi (*Aspergillus niger* and *Aspergillus oryzae*) (Fox, 1985). These enzymes are divided into two groups based on their operational pH range. Fungi produce acid pH enzymes which are used in the production of acid whey and acid whey permeate. The enzymes from yeasts and bacteria have a neutral pH and are used in the processing of milk and sweet whey. Some

other factors that affect the selection of the appropriate enzyme for commercial processing are temperature stability and inhibition by reaction products. The lactase enzymes that are used in the production of hydrolysed milk are obtained from the yeasts (*K. fragilis* and *K. lactis*). The potassium and magnesium ions in the milk are needed for activation of the enzymes (Mahoney and Adamchuk, 1980). The enzymes are inhibited by high levels of calcium and small levels of sodium in the milk. The optimum temperature for these enzymes is around 30 to 40 °C which permits microbial growth (Fox, 1985). Thus, hydrolysis should take place within 2-3 h. The enzymes can be used at a lower temperature (4-6 °C) and for a longer period of time (16-24 h) to minimize the spoilage of the milk. Due to poor heat stability and optimum temperature range, these yeast formed lactase enzymes are mainly used in batch processes.

There are different enzymatic techniques that may be utilized in the production of hydrolysed fluid milk (Zadow, 1986). The different techniques are single- use systems, lactase re-use systems, and immobilised enzymes. In the single use system the fresh milk is heat treated and the milk is cooled down, usually below 10°C, and the enzyme is added to the milk and left to hydrolyze for a fixed period of time. To avoid undesirable microbial growth the milk is usually held at 10°C for an overnight storage. Throughout this process, the milk is being agitated at intervals to keep it homogeneous. After the enzyme activity has terminated, the milk can be further processed or packaged. Modifications to this procedure have been made where the enzymes are added to the milk after pasteurization and just before packaging. This process allows hydrolysis to take place while the product is in transit to the consumer.

In lactase re-use systems hydrolysis is carried out on a protein free stream such as the permeate from ultrafiltration of milk or whey (Zadow, 1986). The enzyme is recovered by ultrafiltration and remixed with milk or whey retentate.

Immobilized enzyme systems are another method of hydrolyzing lactose (Zadow, 1986). This method has the greatest potential for large scale application for the

hydrolysis of milk This process reduces enzyme costs because it is able to operate on a continuous basis. The enzyme is held stationary in a reactor system by physical and chemical binding to an organic or inorganic support.

There are some advantages and disadvantages of using the lactase enzyme in lactose hydrolysis (Repelius, 1983). One advantage is the lactase enzyme does not alter the nutritional quality of a product (Anonymous, 1984). Another advantage of using the lactase enzyme in hydrolysis is an increase in sweetness (Zadow, 1993). Lactose hydrolysed milk requires less sugar to be added to milk drinks because of the production of glucose and galactose which are sweeter than lactose (deVrese, 1993). Also, hydrolyzed lactose has increased solubility (Mitchell and Hourigan, 1993; Repelius, 1983). The solubility of lactose in its unaltered state is about 18% while the solubility of hydrolyzed lactose is around 50%. This is beneficial in preventing lactose crystal formation in frozen desserts. Increased browning due to the production of glucose and galactose are also advantageous in the bakery industry (Mitchell and Hourigan, 1993).

Commercial forms of lactase are also available to consumers as tablets or liquid drops (Sinden and Sutphen, 1991). The tablet form taken before eating a food contains 3,000 Food Chemical Codex (FCC) units of lactase. The liquid form of the enzyme can be added under refrigeration conditions to milk (5 to 7 drops/qt) 24h prior to drinking to cause 70% hydrolysis.

Lactose reduced milk, cottage cheese, American cheese slices, and ice cream are being manufactured to meet the needs of the lactose intolerant population (Reiter, 1991). Manufactured lactose reduced products have some consumer appeal but have problems maintaining shelf space in retail food supermarkets due to low turnover rate compared to regular products. There are also some successful lactose-reduced products on a more global scale; such as hydrolysed pasteurized milks in Toronto and the UK (Zadow, 1993).

Potential Nutritional Concerns of Lactose Reduced Products. Lactose hydrolysed products may affect dental health (deVrese, 1993). The lactose, glucose, and

galactose from hydrolysed milk serve as fermentable substrates for bacteria in the mouth. The fermented substrates produce organic acids which lower the pH of the dental surface, with pH values below 5.5 being damaging to teeth. Milk is considered to be non-cariogenic but hydrolysis of lactose into glucose and galactose increases its cariogenic capacity.

Lactose hydrolysis may also have some effect on the formation of cataracts (Birlouez-Aragon, 1993). Recent studies provide data which states that hydrolysed lactose milk products are not harmful to young adults who metabolize galactose. However, lactose hydrolysed milk products may affect elderly people and people with diabetes. Lactose hydrolysed products may increase a diabetics already high plasma galactose levels even higher which aid in deteriorating their lens transparency. Consequently, there have been a number of contradicting studies that the consumption of lactose hydrolysed products do not cause problems with high levels of blood galactose (Zadow, 1986). There have been studies that show the ingestion of galactose alone leads to elevated blood serum galactose levels, but if galactose is ingested with equimolar parts of glucose there is virtually no increase in galactose levels (Williams, 1983).

Nutritional Value of Milk. In the US, dairy products, with the exception of butter, provide 72% of calcium, 41% of riboflavin, and 21 % of the protein (Heller, 1988). Milk is also considered to be a very high quality protein with a balance of essential amino acids. The FAO/WHO scoring pattern for relative protein quality of different food gives bovine milk a score of 95 with human milk and whole egg having a score of 100 (FAO/WHO, 1973). Milk is also a source of riboflavin, phosphorus, thiamine, niacin, vitamin A, and fortified with vitamin D (McWilliams, 1993). These nutrients aid in performing many different functions such as building and repairing body tissue, supplying energy, helping build bones and teeth, aid in blood clotting, helping keep skin healthy, protecting against night blindness, and helping prevent anemia (Heller, 1988). Prolonged deficiency of calcium can be associated with osteoporosis, loss of jaw

bone, oral health problems, and hypertension. Osteoporosis a painful bone disease has been estimated to affect 15 million Americans to some degree, affecting one out of every four Caucasian women over 60 years old.

Even though milk provides essential nutrients needed by the body, there are some nutrients that play a role in creating a bad image of dairy products (Varnam and Sutherland, 1994). In whole milk (3.25 - 3.5 % fat), the level of milk fat, the saturated fatty acids and the cholesterol content are major concerns for the health conscious consumer. High dietary fat intake is associated with obesity (Varnam and Sutherland, 1994).

Skim Milk. The fat content in milk can be lowered by centrifugal separation of milkfat from the serum phase (McWilliams, 1993). Skim milk is milk which has a milkfat reduction and contains 0.5 % or less milkfat (CFR, 1994). Skim milk (also called fat-free) is still an excellent source of nutrients. Vitamin A must be added to skim milk because the fat-soluble vitamins are removed with the fat (Potter, 1986). Skim milk is ideal for individuals who are trying to control calories and cholesterol (McWilliams, 1993).

Effect of Heat Treatment on Milk. There are two types of milk proteins serum proteins and caseins (Varnam and Sutherland, 1994). Heat causes serum proteins to denature and precipitate (McWilliams, 1993). The α -lactalbumins and B-lactoglobulins precipitate when heated; the higher the temperature the faster the denaturation of the proteins. Heat causes these proteins to gradually relax from their tertiary state to a secondary state, exposing sulfhydryl groups. These sulfhydryl groups contribute to the cooked flavor of heated milk. These precipitated proteins interact with lactose causing nonenzymatic browning (Maillard reaction) which gradually causes color and flavor changes. Ordinarily heating of milk in regular food preparation does not affect casein micelles when the pH of the medium is essentially neutral, but the stability of casein does change when heated in an acidic environment. In addition, a severe heat treatment at pH

7 may cause casein to coagulate by causing the release of esterified phosphates and hydrolysis of peptide bonds in the casein molecule (Palmer, 1972).

Scum formation occurs in milk products heated to and held above 60 °C because the denatured proteins join together (McWilliams, 1993).

Factors Affecting Milk Shelf-life. Functional properties of milk are affected by the sequence of amino acids and the conformational structure of the proteins (Varnam and Sutherland, 1994). The storage life of milk is based on the rate and effect of chemical changes during storage. Oxidation and lipolysis cause major chemical changes that effect the shelf-life of the milk. During oxidation, peroxidases are formed by the reaction of oxygen and unsaturated fatty acid esters. These peroxidases decompose to yield carbonyls which produce oxidized flavors in milk. Lipolytic rancidity is the release of free fatty acids following the hydrolysis of the triacylglycerol molecule. Heat stable enzymes from microbial origin are a major cause of lipolytic rancidity. Lipases and proteases have a high heat resistance and withstand pasteurization, but are of little importance in the spoilage of pasteurized milk due to milks short refrigerated life (Varnam and Sutherland, 1994).

3. Milk and Egg Mixture

Products Processed with Milk and Eggs. There are a variety of products that contain milk and eggs as key ingredients. Milk and eggs can be used in many formulations ranging from frozen desserts, beverages, quiches, puddings, and custard dishes.

Frozen egg custard contains the same ingredients as ice cream with the addition of 1.4% minimum egg yolk solids (Arbuckle, 1986). Egg nog ice cream is also another frozen dessert that contains plain ice cream mix, dry egg yolks, vanilla extract, lemon extract, rum, egg color and spices as desired.

Egg nog is a beverage which is usually a consumed during the holiday season. Egg nog as defined in the Pasteurized Milk Ordinance must contain not less than 8.25%

milk solids not fat (MSNF), 6% milk fat, and 1% egg yolk solids content (U.S. Dept. Health and Human Services, 1993) The milk source can be milk, cream, partially skimmed milk, or skim milk used alone or in combination. The egg yolk source can be a liquid, frozen, or dried egg yolk, or liquid, frozen, or dried whole eggs, and one or more of the ingredients with liquid or frozen egg white. Some of the sweeteners that can be used in the product are sucrose, invert sugar, brown sugar, high fructose corn syrup, and maltose. Other optional ingredients that may be used are salt, flavoring ingredients, stabilizers, and color additives that do not impart the color of eggs (U.S. Dept. Health and Human Services, 1993).

Custards are a baked or stirred mixture consisting of milk and eggs and usually flavored with salt and vanilla (Thomas and Coulter, 1970). The eggs, which are readily available and inexpensive, bind the custard ingredients together and provide high quality protein and a variety of vitamins and minerals. Custards are usually tender and easily digested (American Egg Board (AEB), 1981). They are a good first dessert for babies, and a nutritious dessert for the ill or elderly. Over the years, custards have come in many varieties ranging from plain to very fancy (Schaeli, 1992). The first written recipe for plain baked custard pie was credited to the Amish (AEB, 1981).

There are several different types of custard products that can be made just by changing a few simple ingredients (Schaeli, 1992). Flan is a dessert type baked custard topped with caramel (AEB, 1981). Custard pies can be flavored with a list of different fruits such as pumpkin, blueberry, and apple. Puddings are another dessert which closely favor the custard. Puddings usually contain the basic ingredients of milk and eggs along with different flavoring. For example, rice pudding is a dessert. Rice is cooked in milk and usually enriched with candied fruits, eggs, and whipped cream. Bread pudding is another type of pudding which utilizes day old bread and a custardy mixture. Bread pudding can be flavored with a variety of fruits or chocolate. It is usually cut into squares and served warm with or without a topping. Custard type mixtures can be used to make

English trifle, which consists of a creamy custard layered with sponge cake and fresh fruits. Bavarian cream is another light dessert in which gelatin and whipped cream are used to firm the custard.

Custard can also be used as a main entree (AEB, 1981). For example, quiche is a unsweetened baked custard flavored with a particular meat, cheese, fish, vegetables, or a combination of these ingredients. Strata is another baked entree that uses a unsweetened custard base and bread. An alternative version of strata is a layered dish of custard base with meat, cheese, and/or vegetables. Besides custard type products, the milk and egg mixture could be used in other applications where milk and eggs are the major ingredients in a product (Duncan, 1997). A product called Lacto- pudding is similar to the traditional custard mixture of milk, eggs, and flavoring was formulated in the Phillipines (Mann, 1981). This dairy dessert is based on skim milk, sugar, maize starch, gelatin, eggs, food coloring, and vanilla.

Thermal Processing. Heat treatments are used in food processing to eliminate pathogenic and toxin producing microorganisms that may endanger public health safety and/or eliminate microorganisms that may cause food spoilage during distribution and storage (Stumbo, 1973). There are two main categories of heat treatments used to reduce the microbial population of a food. These heat treatments are pasteurization and sterilization.

Pasteurization is a relatively mild heat treatment whose main objective is eliminating non-spore forming pathogenic organisms, and inactivating enzymes, and reducing the load of spoilage organisms in a food (Frank, 1997). Pasteurized food must be stored at refrigeration temperatures to prevent the growth of spores that survived the heat treatment. The shelf life of pasteurized foods depends on the type of food and the storage conditions. A relatively new method of pasteurization for foods (i.e. milk) is ultra - pasteurization. Products that have been ultra- pasteurized have been thermally processed at or above 138 °C for at least 2 sec before or after packaging to produce a

product with an extended shelf life under refrigerated conditions (CFR, 1994).

Sterilization, referred to as commercial sterility in the food industry, is the application of a heat treatment, higher than normal pasteurization, capable of rendering a food free of microorganisms having public health significance and capable of reproducing under non-refrigerated conditions of storage (U.S. Dept. Health and Human Services, 1993).

In foods there are several substances that may have an affect on the heat resistance of microorganisms. These substances, include protein, fat, salts, and carbohydrates. Foods with high protein contents require a more intense heat process than a low protein food to achieve the same results (Stumbo, 1973). Also, products with colloidal size particles require a more intense heat treatment. Fat is believed to increase the heat treatment by affecting the cell moisture and making the microorganisms more heat resistant. A study of the effect of long chain fatty acids on *Clostridium botulinum* indicate that long chain fatty acids are better protectors of microbes undergoing heat treatment than short chain fatty acids (Suigyama, 1951). Salt, depending on the kind of salt and the concentration used, may have a varied effect on the heat resistance of microorganisms (Jay, 1992). Sugars in a product increases the required temperature applied to a product. A high concentration of sugar in a product lowers the water activity and increases the heat resistance of microorganisms.

Destruction of Microorganisms. In order to apply an appropriate heat treatment for the destruction of microorganisms there are some important concepts that must be understood (Jay, 1992). The thermal death time is the time needed to kill a given number of organisms at a specified temperature. This method requires the temperature to be kept constant so that the time necessary to kill all organisms can be determined.

The D value, also called the decimal reduction time, is the time in minutes at a specified temperature to destroy 90% of the organisms in a population. The “z” value is the degrees (in Fahrenheit) required for the thermal destruction curve to traverse one log

cycle. In general terms, the D value shows the resistance of an organism to a specific temperature, whereas the z value gives information on the relative resistance of an organism to different destructive temperatures. The D and z value allows one to calculate an equivalent thermal process at different temperatures. This is of value when developing an appropriate thermal process for a new product. A listing of some of the microorganisms of concern in the pasteurization of food, namely milk and eggs, is given in Table 1.

Generally, the number of organisms present in a product or the specific types of organisms present prior to pasteurization is not known (Potter, 1986). Thus a considerable margin of safety is applied to ensure that the minimum heat process should reduce the probability of the survival of the most resistant spores. In the canning industry, the 12D process is applied to low acid foods so that the minimum heat process should reduce the probability of the survival *Clostridium botulinum* spores (Jay, 1992).

Thermal Processing of Milk and Eggs. In order to make sure products that contain milk and eggs are safe for consumption, pathogens and spoilage organisms must be destroyed by pasteurization (Banwart, 1989). The pasteurization of milk was previously designed to destroy *Mycobacterium tuberculosis* which was believed to be the organism of chief concern in milk. However, a study by Enright et al. (1956) showed that *Coxiella burnetti* is more heat resistant than *Mycobacterium tuberculosis*. They reported that the average maximum resistance of *Coxiella burnetti* in milk at $D_{150} = 0.50$ to 0.60 min and $z = 8$ to 10 °F whereas *Mycobacterium tuberculosis* has a $D_{150} = 0.20$ to 0.30 min and $z = 8$ to 10 °F. *Coxiella burnetti* are gram negative short rods, but may appear gram positive under certain conditions. These organisms are shed in raw milk through infected cows. These organisms do not grow outside a host cell but can be carried in food infecting humans with Q fever. Accordingly, pasteurization of milk and milk products has been designed to destroy *Coxiella burnetti*. The pasteurization temperatures also destroy yeasts, molds, gram-negative bacteria, and many gram positive organisms

(Penfield and Campbell, 1990). However, organisms that can survive these high temperatures, but not necessarily grow are *Streptococcus* and *Lactobacillus* species (Banwart, 1989). These organisms will eventually cause spoilage. The Pasteurized Milk Ordinance (PMO) gives several temperature and time combinations used in the pasteurization of milk and milk products (U.S. Dept. Health and Human Services, 1993) (Table 2).

Table 2.1. Comparative heat resistance of bacteria for pasteurized foods.

Bacterial Groups	Approximate range of heat resistance	
	z	D ₁₅₀
Pathogenic Microorganisms		
<i>Mycobacterium tuberculosis</i>	8-10	0.20-0.30
<i>Coxiella burnetti</i>	8-10	0.50-0.60
<i>Salmonella spp.</i>	8-10	0.02-0.25
<i>Salmonella senftenberg</i>	8-12	0.80-1.00
Spoilage Microorganisms		
Non-spore forming bacteria, yeasts, and molds	8-12	0.50-3.00

(Stumbo, 1973)

Table 2.2. Temperature and time combinations for milk pasteurization

Temperature °C	Time
*63	30 min
*72	15 sec
89	1.0 sec
90	0.5 sec
94	0.1 sec
100	0.01 sec

* If the fat content of the milk product is 10 percent or more, or if it contains added sweeteners, the specified temperature shall be increased by 3°C (5°F)(U.S. Dept. Health and Human Services, 1993).

Eggs are a source of salmonella (Defigueiredo et al., 1976). Fresh eggs are seldom a food borne disease problem. However, processed eggs may be held for various periods at room temperature which could allow salmonella growth. Normal processing conditions for drying or freezing is seldom sufficient to destroy salmonellae. Thus, the rules and regulations given by the USDA require all frozen, liquid, and dried whole egg, yolk, and white to be pasteurized or treated to destroy salmonellae (Banwart, 1989). Dried eggs heated at 57 - 60 °C for 7 -14 days destroys salmonella. Destroying salmonella in liquid eggs is more difficult. The organism *Salmonella senftenberg* 775W is reported to be more resistant to moist heat. Salmonellae in liquid egg yolk is more resistant than in whole egg (Cunningham, 1995). The increased resistance of egg yolk is due to the lower pH and higher solids content. A patent by Swartzel (1986) developed a method of ultra pasteurization of liquid whole eggs using temperatures ranging from 63.7 to 72.2 °C for 2.7 to 192.2 sec. This process achieved shelf lives ranging from 4 to 24 wks at 4 °C. Functionality requirements often limit the extent of heat treatment (Defigueiredo, 1976). Egg white has a pH of about 9.0. Pasteurization of egg white under conditions of 60°C to 62°C for 3.5 to 4.0 min causes coagulation of proteins. However, when the pH of egg whites is dropped to about 7.0 the egg white proteins are more heat stable. The addition of hydrogen peroxide, aluminum salts, or iron salts increases the stability of the egg protein conalbumin during pasteurization at 60 to 62 °C. Table 3 gives the pasteurizing temperatures for liquid egg products (Banwart, 1989).

Thermal Processing of a Milk and Egg Mixture. There are several different products that the dairy industry is currently processing which contain milk and eggs as primary ingredients. Egg nog is one of these products that has specific thermal processing guidelines stated in the PMO. There are specific temperature and time guideline stated in the PMO for the pasteurization of egg nog (Table 4) (U.S. Dept. Health and Human Services, 1993).

Table 2.3. Pasteurization conditions for egg products

Product	Temperature (°C)	Average Holding Time (min)
Whole egg, plain	60	3.5
Yolk		
Plain	60 or 62.2	7.0 or 3.5 (respectively)
Sugared or Salted	62.2 or 64.4	7.0 or 3.5 (respectively)
Egg white		
Plain, pH 9	56.7	3.5
Plain, pH 9, treated w/ H ₂ O ₂	51.7	3.5
Stabilized with (Al ₂ (SO ₄) ₃ pH 7	50	3.5

(Banwart, 1989)

Table 2.4. Temperature and time combinations for egg nog pasteurization.

Temperature °C	Time
69	30 min
80	25 sec
83	15 sec

(U.S. Dept. Health and Human Services, 1993)

A United States patent was given for the process of producing a sweet custard foodstuff with a long term shelf life based on milk and eggs (U.S. patent 4877625, Dieu and CuQ, 1989). The product contained whole egg concentrates, milk, sugar, modified starch, stabilizers, and colorants. The process included mixing the above ingredients at a temperature between 10 - 40°C and preheating the mixed mixture between 60 - 90°C. The mixture was sterilized by direct steam injection with temperatures between 100 - 160°C and a hold time between 1 to 10sec. The product was then initially cooled between 60 - 90°C by evaporation of the water injected as steam. Next, the product was homogenized before being finally cooled down to 15 - 30°C. The product was then aseptically filled and stored at room temperature. This process did not alter the quality of the product and also extended the shelf life of this milk and egg based product for an average of six months.

Problems with Processing Milk and Egg Mixture. In processing certain food products there may be problems depending on the type of equipment used in the process. In thermally processing a milk and egg mixture, fouling may be a problem. Fouling is the accumulation of deposits on a heat transfer surface that reduces the efficiency of the heat treatment (Walker, 1990). Fouling can cause a loss of heat transfer performance. Fouling can be related to the quality of a product in terms of its microbial content, color, texture, and taste (Bott, 1995). Heat exchangers are used to heat the food to cook it, reduce its volume by evaporation, or kill microorganisms to increase the shelf-life and render the food safe for consumption. The deposits from fouling are the denaturants of the food.

Egg and milk proteins are sensitive to heat. Mineral deposits and protein denaturation play a role in milk fouling. With temperatures below 110°C a milk film deposits can form consisting of about 50 - 60 % protein and 30 - 35 % mineral material (Burton, 1968). In milk the heat sensitive proteins are immunoglobulins, β -lactoglobulin, and α -lactalbumins, with β -lactoglobulin being partially responsible for the milk film formation. Calcium phosphate makes up a large part of the mineral deposits. Protein fouling is dependent on reactions involving sulfhydryl and disulfide bonds in the protein (Lalande and Rene, 1988). Milk fouling occurs when the protein, a three dimensional structure is distorted by the heat (Bott, 1995). The proteins are held together by intramolecular disulfide bonds, ion pair interactions, and van der Waals forces. During denaturation, the proteins unfold from their tertiary and secondary structure allowing more of its inner structure to be able to react with other molecules causing insoluble lumps of protein to form which induce fouling (Fryer and Pritchard, 1989). These reactions are significant at temperatures above 65°C. Yet, for the pasteurization of milk it is not possible to operate at such low temperatures (Bott, 1995).

In the food industry, the type of heat exchanger selected to process a product is critical to the appearance and consistency of the product (Donovan, 1992). There are several different types of heat exchangers. The most familiar type of heat exchanger among manufacturers is the plate heat exchanger. This type of exchanger is ideal for processing Newtonian liquids. Normally, non particulate products with low to medium viscosities are processed using this type of heat exchanger. Another type of heat

exchanger is the tubular heat exchanger. Tubular heat exchangers have no contact points, unlike the plate heat exchanger. This feature allows the processing of products with very high concentrations of suspended solids without the risk of blockage. These units are useful when processing products in which the integrity of the particles may be compromised. Another type of heat exchanger is the scraped surface heat exchanger. This type of heat exchanger is often used as a last resort by most manufacturers. These heat exchangers are suitable for products which are viscous and sticky.

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CHAPTER III

THERMAL PROCESSING OF SWEETENED AND UNSWEETENED MILK AND EGG MIXTURE

A. Abstract

A milk and egg mix was processed at 96°C with a 10 sec hold time and was evaluated for nutritional composition and functionality. Both sweetened and unsweetened formulations were evaluated. The process was more than sufficient to destroy *Coxiella burnetti*, the most heat resistant organism of concern in processing milk, and *Salmonella senftenberg*, the most heat resistant organism of concern in processing eggs. The spoilage organisms received a 2,200 D process which was more than adequate for providing a relatively safe product.

The nutritional profile of the milk and egg mix was improved when dried eggs (solids and liquid proportion equivalent to whole egg) and whole fat milk were replaced with dried egg white, cholesterol reduced egg yolk, and skim milk. The fat and cholesterol were reduced in the sweetened mixes by 33% and 44%, respectively, in the cholesterol reduced formulation (CRF) when compared to the control formulation (CF). The fat and cholesterol were reduced in the unsweetened mixes by 29% and 37%, respectively, in the CRF as compared to the CF. The protein content of the milk and egg mix was not altered by the utilization of cholesterol reduced egg yolk in the CRF when compared to the CF. Addition of β -galactosidase decreased the lactose for sweetened and unsweetened mix by 89% and 94%, respectively. The CF was more yellow than the CRF in the mixes and baked gels of the sweetened and unsweetened formulations ($p < 0.05$). There was also no difference in gel strength between the baked gels made from the two formulations for either the sweetened or unsweetened mix. However, the CF mix was more viscous than the CRF mix for both the sweetened and unsweetened formulation.

B. Introduction

Americans have high expectations (Christian and Greger, 1994). They want safe, convenient, nutrient rich foods at an economical price. Over the last decade the number of convenient processed foods has soared. These processed foods have been enhanced by hindering spoilage and promoting safety, nutrition, palatability, and convenience.

Product safety is a major factor in consumer food selection. The major public health problems associated with the food supply are microbial contamination of food (Christian and Greger, 1994). In 1988, an expert panel from the Institute of Food Technologists estimated that between 24 and 81 million cases of food borne diarrheal disease occur each year in the U.S. by microorganisms. The outbreaks cost between \$5 and \$17 billion in medical treatment and lost productivity (IFT Expert Panel on Food Safety and Nutrition, 1988).

Sweetened products containing milk and eggs are excellent medium for nurturing the growth of organisms responsible for food borne illness (Kaplan, 1993). In 1988 an outbreak of food poisoning in 17 patients was caused by *Salmonella enteritidis* (Barnes and Edwards, 1992). The individuals had consumed custard slices, a sweet milk and egg mixture, that had been purchased from the same bakery. An investigation of the bakery found that uncooked whole shell eggs were the culprit in the outbreak of *Salmonella enteritidis*.

Incidences such as these have made consumers more aware of product safety when purchasing foods. This concern is warranted because of consumers' lack of time, and their increased demand for convenience; they are utilizing the foodservice sector more.

The foodservice sector has experienced rapid increased growth. In 1995, \$280 billion in sales (excluding taxes and tips) occurred within the food service sector with commercial food service industries (i.e. restaurants) rendering 79% of the sales (Price, 1996). There is an opportunity for the dairy industry to take advantage of this trend by providing the foodservice industries with more nutritionally enhanced dairy products,

thereby increasing market share (Duncan, 1997). Attention must be given to the appropriate processing to ensure safety while retaining the desired quality characteristics of the new products.

The primary objective of this study was to formulate and adequately heat process a milk and egg mix to ensure safety to consumers while retaining the functional and physical integrity of the product. The secondary objective of this study was to formulate a nutritionally enhanced version of the milk and egg mix that was reduced in fat, cholesterol, and lactose while retaining a high quality of nutrients such as protein and calcium.

C. Materials and Methods

1. Mix Formulations and Preparation

Formulations. Fluid milk and egg mix formulations for sweetened and unsweetened applications were evaluated separately. In each experiment, two formulations with two levels of lactose were tested in a split plot design (whole plot factor = formulation; split plot = lactose hydrolysis). A baked custard formulation by Penfield and Campbell (1990) was modified and used to develop the formulations (Table 5). In all formulations whole milk was replaced 1:1(vol/vol) with skim milk. The experimental product was further modified to reduce cholesterol and fat (cholesterol reduced formulation = CRF). The control product (control formulation = CF) was reformulated to replace fresh egg with dried egg white solids (Henningsen P-21, Henningsen Foods, Inc., Omaha, NE) and dried egg yolk solids (Henningsen Y-2, Henningsen Foods, Inc., Omaha, NE). The dried egg contributed the equivalent solids of a fresh whole egg. The dried egg was reconstituted with skim milk to the equivalent moisture content of fresh egg. Dried egg white solids (Henningsen P-21, Henningsen Foods, Inc., Omaha, NE) and reduced-cholesterol, reduced-fat liquid egg yolk (SourceTM cholesterol and fat reduced egg yolk, Source Food Technology, Inc, Burnsville, MN) were used in the experimental product (CRF). Additional skim milk was added to the CRF to be equivalent to the moisture found in fresh egg white. Replacing the fresh eggs

with the modified egg products in the original proportions did not produce a desirable gel strength (preliminary work). Thus, a dairy stabilizer (Dariloid 400, NutraSweet Kelco Co., San Diego, CA) used in liquid systems processed by HTST / UHT systems was used to enhance gel strength.

Preparation. Procedures described by Penfield and Campbell (1990) for mixing and baking custard were modified and used for preparation of sweetened and unsweetened type custards (Wu,1996). The formulation was scaled up to 16 times the standard

Table 3.5. Formulation for sweetened¹ milk and egg mixture.

Ingredients (g)	Formulations			
	CF ² - LR ³	CF- NLR ⁴	CRF ⁵ -LR	CRF- NLR
SM ⁶ (mL)	287.1	287.1	278.5	278.5
DEW ⁷	6.05	6.05	6.05	6.05
DEY ⁸	6.90	6.90		
RCEY ⁹			15.5	15.5
Sugar	25.0	25.0	25.0	25.0
Salt	0.3	0.3	0.3	0.3
Stabilizer ¹⁰	0.59	0.59	0.59	0.59
Vanilla (mL)	1.20	1.20	1.20	1.20

¹ The unsweetened formulations were the same with the exemption of sugar and vanilla

² CF = Control Formulation

³ LR = Lactose reduced

⁴ NLR = No lactose reduction

⁵ CRF = Cholesterol reduced formulation

⁶ SM = Skim milk (Richfood skim milk, Richmond, VA)

⁷ DEW = Dried egg white (Type P-21, Henningsen Foods, Inc., White Plain, NY)

⁸ DEY = Dried egg yolk (Type Y-2, Henningsen Foods, Inc., White Plain, NY)

⁹ RCEY = Reduced cholesterol, reduced fat liquid egg yolk (Source Food Technology, Inc., Burnsville, MN)

¹⁰ Stabilizer = (Dariloid 400, Nutrasweet Kelco Company, San Diego, CA)

formulation (Penfield and Campbell, 1990) in order to make batches of approximately 4.5 L for processing. Milk used in the formulation was preheated to 30 °C . While milk was heating, other ingredients (control= dried egg white, dried egg yolk, sugar, salt, and stabilizer; experimental= dried egg white, reduced-cholesterol, reduced-fat liquid egg yolk, sugar, salt, and stabilizer) were mixed together. A portion (control, 593.6 mL ; experimental, 456 mL) of the preheated milk was added to hydrate the dried eggs and other ingredients and mixed for about 15 sec. The vanilla and remaining 4 L of milk were gradually added in 1L aliquots to the mixture and mixed using a household type hand held mixer (Type 4172-B, Braun, Mexico) for approximately 4 min.

Thermal Processing. Thermal processing parameters were determined during preliminary laboratory experimentation. Dieu and CuQ (1989) (*Process For Producing A Sweet Custard Foodstuff With A Long Term Shelf Life Based On Milk And Eggs*) suggested processing parameters that were modified for a high temperature short time (HTST) process. A laboratory scale pasteurizer (Microthermics UHT/ HTST Lab 25 - DH, Microthermics, Inc., Raleigh, NC) was used in the thermal processing. This type of unit is a tubular heat exchanger. The product enters the system through a product tube and goes through two tubular product heaters (Microthermics, 1992). The temperature of the product in these heaters is controlled by adjusting the steam pressure, used to generate the hot water, and the quantity of hot water bypassing the hot water generator (Microthermics, 1992). The product is cooled within the system by the use of chilled water. The system was operated at a flow rate of 1200 mL/min with a 10 sec hold time. Thermocouples were placed throughout the processing path (Table 6) so the different temperature changes within the heating line could be monitored and recorded.

Table 3.6. The unit placement of thermocouples between sections of the heating line.

Thermocouple	Unit Placement
1	Pump outlet - Preheater inlet
2	Preheater outlet - Heater 2 inlet
3	Heater 2 outlet - Hold tube inlet
4	Hold tube outlet - Cooler inlet
5	Cooler outlet - Product outlet

The temperatures were recorded and retrieved using a Campbell Scientific 21X Micrologger and Campbell Scientific PC208 software (Logan, UT). The product entered the system with a temperature of ~ 30°C before entering the preheater which was set at a temperature of 37°C. The mix continued to flow through heater no. 2, set at a temperature of 104°C, before entering the hold tube and held at a temperature of 96°C for 10 sec. The product then reached the cooler set at a temperature of 21°C, for product cool down. Before exiting the product outlet the product was cooled down to ~ 29°C and collected into sterile containers.

Homogenization. Following pasteurization, the mix was pumped through a sanitized homogenizer (APV Gaulin Model 15 MR, Everett, MA) at a total pressure of 2500 psi (2000- 1st stage; 500- 2nd stage) to break down the fat globules and other particles and create a homogenous mixture. Two sterile (1 L) volumetric flasks were used to collect the mix from each formulation. The remaining mix was collected in a large plastic vessel and refrigerated at 4°C for any additional baking studies.

Enzyme Addition. Mix was refrigerated at 4°C for 4 h. β -galactosidase enzyme (3.5 mL; Lactozym™ 3000L, Novo Nordisk, Franklinton, NC) was filtered through a sterile 0.45 μ m acrodisc (Gelman Sciences prod. no 4184, Fisher Scientific, Pittsburgh, PA) and added to one - 1 L flask of each mix. The other 1 L mix of each formulation

served as the non-enzyme treated control. The mix was then stored at 4°C for 72 h to achieve an 80% minimum reduction in lactose.

Baked Gel Preparation. Eighty grams of mix was poured into a (90 g) glass custard cup; three gels were prepared with each formulation. The cups were placed in a water bath and baked for 35 min at 177°C. Gels were cooled to room temperature, covered with plastic film, and stored at 4°C until analyses.

2. Chemical Analyses

Lactose and Galactose Concentrations. Lactose and galactose concentrations were measured spectrophotometrically on mix that had been stored for approximately 72 h at 4°C. For analysis, 2 mL of custard mix were added to 20 mL of 12% (w/v) trichloroacetic acid, mixed, and centrifuged at 6000 rpm for 20 min (Davidson, 1995). The pH of the clear supernatant (10 mL) was adjusted to a neutral pH of 7.0 with 1N NaOH and 0.1N NaOH and diluted to 25 mL with H₂O. Lactose and galactose concentrations were assayed with the Boehringer-Mannheim Test Kit (Gene-trak, Framingham, MA) and absorbance measured spectrophotometrically (Model 1001, Fisher Scientific, Inc., Pittsburgh, PA) at a wavelength of 340 nm. Duplicate measurements were taken on each mix sample.

Protein Concentration. Samples were stored at 4°C for approximately 36 h prior to analysis. Approximately 500 mg of custard mix from each of the different formulations was used in the protein analysis. The different sample formulations were measured in duplicate. Association of Analytical Chemists (AOAC) method 24.024 was used to determine nitrogen content, and the 6.25 conversion factor for protein was used (AOAC, 1984). A Buchii B-343 apparatus (Brinkmann Instruments, Inc., Westbury, NY) was used in the analysis.

Total Fat Concentration. All samples were stored at 4 °C for approximately 36 h prior to analysis. Two grams of mix were used for analysis of total fat. The chloroform and methanol extraction method described by Bligh and Dyer (1959) was used to

gravimetrically determine total fat concentration. Duplicate measurements were made on each sample. Extracted lipid was used for cholesterol and fatty acid analysis.

Cholesterol Concentration. Two milliliters chloroform containing approximately 9-30 mg lipid obtained by the Bligh and Dyer (1959) method for lipid extraction was used to determine the cholesterol concentration. Sample vials that contained lipid amounts closer to the 30 mg amount were divided into two aliquots so that absorbance would fall on the standard curve. The samples were stored for approximately 9 wks at 0°C in sealed vials flushed with nitrogen. The chloroform was removed by a stream of dry nitrogen before continuing with the cholesterol procedure described by Kates (1986). Cholesterol concentrations were measured spectrophotometrically at 550 nm by a uv/vis spectrophotometer (Model lambda 3B, Perkin- Elmer, Norwalk, CT). Serum cholesterol (99%, cat. no. C7921, Sigma, St. Louis, MO) was used to make a standard stock solution containing 1 mg/mL of cholesterol. The stock was used to prepare working standards in the range of 0.05-0.60 mg. The amount of cholesterol in the samples was quantified by using the working standards and reading the samples against a linear regression curve.

Fatty Acid Analysis. Two mL of chloroform containing approximately 10-30 mg of lipid obtained by the Bligh and Dyer (1959) lipid extraction procedure was used in determining the fatty acid methyl esters (FAME). The chloroform and lipid mixture was stored for approximately 9 wks at 0°C in sealed vials flushed with nitrogen. The chloroform was removed by a stream of dry nitrogen before continuing with the FAME analysis. A procedure by Luddy, Barford, and Reinenschneider (1960) was used to methylate the extracted fat. Gas chromatography was used to determine the FAME. A Shimadzu GC14A gas chromatograph (Siesakusho Ltd., Kyotot, Japan) with an Autoinjector (Model AOC-14) and Chromatopac C-R4AX processor was used in data analysis. Flame ionization detection and temperature programming were used for analysis. A 30m x 0.32 i.d SP2330 capillary column (Supelco Inc., Bellefonte, PA) was used to achieve resolution of the long chain fatty acids. The carrier gas was helium at a

flow rate of less than 1 mL/min, and the make up gas flow rate was 50 mL/min. The temperature program had an initial temperature range of 60 to 100°C at 10°C/min with a hold time of two min at 100°C and a final temperature range of 100 to 220°C at 15°C/min and hold time of 12 minutes at 220 °C. The detector and inlet temperatures were set at 230°C. Injections of 1 uL were made with an autoinjector (Model AOC-14). Peak areas were determined by integration and quantification determined against the internal standard, heptadecanoic acid (C:17). The peaks were identified by their retention times against pure standards (AOC No. 1 & AOC No.5, Supelco, Inc., Bellefonte, PA).

Moisture. Percent moisture was performed on mix samples by standard methods using a forced draft oven (Model OV490A2, Blue M Electric Co., Blue Island, IL) (American Public Health Association, 1992). Approximately 5 g of mix were weighed into preweighed nickel dishes. Previously, the nickel dishes had been exposed to 100 °C for 1 h to remove any moisture and cooled in a desiccator before weighing. The 5 ± 0.5 g of mix were placed in a forced draft oven at 100 °C for 3 h. After drying the sample it was placed in a desiccator to cool. Percent moisture was calculated with the following equation:

$$\text{Percent Moisture} = \frac{\text{Weight of sample} - \text{Weight of sample after drying}}{\text{Weight of sample}} \times 100$$

Ash. Five grams of mix used to calculate the percent moisture were used to calculate ash. The Association of Analytical Chemists method 16.035 was used to determine ash by the gravimetric method (AOAC, 1984) . Samples were transferred to a muffle furnace set at 550 °C for approximately 16 h before cooling and weighing. Percent ash was calculated by the following equation:

$$\text{Percent Ash} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3. Physical and Microbiological Analyses

Color. Color measurements of the top and bottom surfaces of baked custard samples were taken at 3 different locations (Minolta Chromameter, Model CR-200,

Minolta Camera Co., Ltd., Osaka, Japan). The custards were removed from the dish to measure the color of the bottom surface. The color measurements were made on the baked custard 72 h after the mix was prepared. The color measurements were expressed as Hunter “L”, “a”, and “b” values. Calibration of the instrument was carried out using a white tiled Minolta Reference tile (L = 97.91, a = -0.68, b= +2.44). Means and standard deviations were calculated by the statistics mode on the chromameter. The color of the custard mix before baking was also evaluated by the same procedure. The mix was measured through a clear glass test tube held in the tube holding apparatus (Minolta Camera Co., Ltd., Osaka, Japan).

Gel Strength. Firmness was measured as the force (g) needed to penetrate an 80 g baked custard sample. A Stevens L.F.R.A. Texture Analyzer (Model TA - 1000, Texture Technologies Corp., Scarsdale, NY) with a TA - 2 cone attachment operated at a speed of 2 mm/s and a distance of 5 mm was used in the analysis. Measurements were obtained from two different locations on the top surface of the custard and the values averaged. The custards were allowed to sit at room temperature for approximately 6 h before measurements were taken.

Syneresis. Syneresis, the drainage of a liquid from a baked custard gel, was measured as described by Penfield and Campbell (1990). The gelled custard sample was inverted on a cheesecloth supported on a funnel. The exudate was collected in a 10 mL graduated cylinder over a one hour period at 22.2 - 23.3 °C and the volume recorded.

Water Activity. Water activity (A_w) was measured on the mix and baked custard gel 72 h after the mix was thermally processed. The water activity was measured by a Decagon CX-1 Water Activity System (Decagon Devices, Inc., Pullman, WA).

Viscosity. Viscosity was measured on refrigerated (7 ± 2 °C) custard mix 72 h after the mix was processed. A Brookfield Synchro-Lectric Viscometer (Model RVF, Brookfield Engineering Laboratories, Inc., Stoughton, MA) with the spindle 2 attachment operated at a speed of 20 rpm was used to measure viscosity. A 600 mL glass beaker was

filled with 450 ml of custard mix for analysis. Two readings per sample were taken, with 5 sec between readings, and averaged.

Aerobic Plate Count. A standard plate count was performed on the mixes following standard methods (American Public Health Association, 1992). Mix was plated immediately after the lactase enzyme addition. One milliliter samples of mix were plated at 10^0 , 10^{-1} , 10^{-2} , and 10^{-3} dilutions on Standard Methods Agar in duplicate and incubated at $32\text{ }^\circ\text{C}$ for 48 ± 3 h. Colony counting was reported by APHA (1992) guidelines.

4. Data Analyses

Data Collection. Three replications consisting of two weeks for each replication were performed on the samples. The first week consisted of analysis that would only be performed on the custard mix samples in the first week. The analysis that were performed were as follows: protein, fat, moisture, ash, fatty acid methyl esters, and cholesterol. Other tests were performed in week 1 and in week 2 keeping the same days for analysis in week 2 as in week 1. These tests were as follows: standard plate count (mix), viscosity (mix), lactose and galactose concentration (mix), water activity (mix & gel), color (mix & gel), texture (gel), and syneresis (gel).

Statistical Analyses. A t- test assuming equal variance was used for the following analyses: percent total fat, percent protein, percent cholesterol, percent moisture, percent ash, percent saturated fat, percent monounsaturated fat, and percent polyunsaturated fat. A split plot design (whole plot factor = formulation; split plot = lactose hydrolysis) was used for analysis on all the tests that were performed on week 1 and week 2. The Statistical Analysis System (SAS Institute Inc., 1988) utilizing the PROC GLM procedure was used to analyze the data collected from the split plot design.

D. Results and Discussion

1. Introduction

The formulation of two milk and egg products, differentiated by the addition of sucrose for sweetening, was completed (Table 5). The sweetened and unsweetened products were evaluated separately for chemical composition, and physical and functional characteristics. The same thermal process was applied to both formulations.

The thermal process for both formulations is discussed first. The results and discussion for the chemical composition and physical and functional characteristics are discussed initially for the sweetened product. Important and unique results from the analysis of the unsweetened products are subsequently discussed.

2. Thermal Processing

A U.S patent for the processing of an extended shelf-life sweet milk and egg based product (U.S. Patent 4877625; Dieu and CuQ, 1989) was used as the basis for setting the processing parameters used. The method of heat treatment from the patent (Dieu and CuQ, 1989) used direct steam injection utilizing a hold temperature between 100 - 160°C with a 1- 10 sec hold time. Preliminary research using a hold time of 10 sec at 100°C in our thermal processing unit was not feasible due to product burn on in the hold tube of the equipment. Through preliminary testing, an adequate temperature (96°C) utilizing the 10 sec hold time was discovered and used as the basis for the processing of sweetened milk and egg mix.

In continuous processing, the time and temperature for destruction of organisms with regard to determining public safety is based on the temperature and time in the hold tube (Swartzel, 1986). The hold tube temperature of 96°C and hold time of 10 sec provided more than adequate D values for the destruction of pathogens and spoilage organisms of concern in milk and egg products. Table 7 compares the reference D values listed in Stumbo (1973) to D_{205} values. The thermal destruction equation (Stumbo, 1973) used to calculate equivalent D values for the thermal process temperature used in this study was as follows:

$$\log D_2 - \log D_1 = \frac{1}{z} (T_1 - T_2)$$

z

D_2 = D value corresponding to temperature T_2 , and the time to destroy 90% of the bacterial population when exposed to temperature T_2 .

D_1 = D value corresponding to temperature T_1 , and the time to destroy 90% of the bacterial population when exposed to temperature T_1 .

Table 3.7. D_{205} values for concern in the milk and egg mix.

Bacterial Groups	Heat Resistance		
	z*	D_{150}^* ¹	D_{205}^* ¹
Pathogenic Microorganisms			
<i>Mycobacterium tuberculosis</i>	10	.3	.000003
<i>Coxiella burnetti</i>	10	.6	.0000019
<i>Salmonella spp.</i>	10	.25	.0000008
<i>Salmonella senftenberg</i>	12	1	.000026
Spoilage Microorganisms			
Non-spore forming bacteria, yeasts, and molds	12	3	.000078

* The larger z and D values (°F) were chosen from the reference values (Stumbo, 1973) to calculate the D values at 205.

¹ 150°F = 65°C; 205°F = 96°C.

In the food processing industry, the number of organisms present in a product is generally not known; thus a margin of safety is employed to ensure a safer product (Potter, 1986). The most pathogenic organisms of concern in this study are *Coxiella burnetti* in milk and *Salmonella senftenberg* in eggs. To ensure a minimum process for safety concerns, the dairy industry applies a 6 D process to *Coxiella burnetti* when pasteurizing milk at 72°C for 15 sec (HTST process) (U.S. Dept. Health and Human Services, 1993). At 65°C, a 6 D process for *Coxiella burnetti* would be 0.1 min; a 6 D process at 96°C for this organism would be 3.0×10^{-7} min. The USDA (1969) requires

that pasteurization assures a 9 D reduction of *Salmonella* in liquid whole egg. At 65°C, a 9 D process for *Salmonella senftenberg* would be 0.1 min; a 6 D process at 96°C for this organism would be 2.9×10^{-7} min. Spoilage organisms are usually more heat resistant than most pathogens. In this study, thermal process of 96°C for 10 sec provided a 89,000 D for *Coxiella burnetti*, a 6,538 D for *Salmonella senftenberg*, and a 2,200 D for spoilage organisms. Therefore, this thermal process provided more than an adequate process for safety.

3. Chemical Composition of Sweetened Formulations

Proximate Analysis. One objective of this research was to develop a nutritionally enhanced milk and egg mixture. Proximate analysis demonstrated a decrease in fat, carbohydrates, and calories in the cholesterol reduced formulation (CRF) as compared to the control formulation (CF) (Table 8). There was a 33% reduction of fat in the CRF. However both formulations have less than 1 g of fat /100 g of milk and egg mix. A one hundred gram portion of custard mix has 3.8 g of fat as listed from food value portions provided in Pennington and Church (1985). Wu et al. (1996) reported that custard mix made with whole milk and fresh eggs has 5.1 g of fat / 100 g. Therefore, replacing whole milk and whole eggs with skim milk and cholesterol and fat reduced egg yolk caused a considerable reduction in fat content. The higher calories and fat in the CF are attributed to the type of egg yolk used in the formulation. The CF had dried egg yolk while the CRF used a cholesterol reduced (CR) and fat reduced (FR) liquid egg yolk. Both treatments were formulated so that the yolk was proportional to egg yolk solids and moisture level were equivalent to the yolk in a fresh egg. Moisture content was slightly higher in the CRF as compared to the CF (Table 8). This increase in moisture is relatively negligible though and should have limited implications with regard to chemical composition, functionality of product or thermal processing.

The total protein concentration and ash are not significantly different between the CF and CRF ($p \geq .05$) (Table 8). The milk and egg sources both provided the proteins in

the mix. Milk and eggs provide the proteins that best meet human needs (Christian and Greger, 1994). The protein concentration in lowfat milk is around 3.3 g/100g (Penfield and Campbell, 1990). A whole egg (50 g) contains 6.25g of protein (AEB, 1989). A 100 g portion of custard mix has 3.8 g of protein as listed in the food value portions (Pennington and Church, 1985). Wu et al. (1996) reported that custard mix made with

Table 3.8. Means¹ ± standard deviations for proximate analysis of sweetened milk and egg mixture.

Constituent (g/100g)	Formulations	
	CF ⁴	CRF ⁵
Fat ¹	0.87 ± 0.13 ^a	0.58 ± 0.13 ^b
Protein ¹	4.87 ± 0.34 ^a	4.98 ± 0.09 ^a
Moisture ²	79.21 ± 0.48 ^a	80.76 ± 0.39 ^b
Ash ²	0.98 ± 0.03 ^a	0.96 ± 0.04 ^a
Carbohydrates ³	13.93 ± 0.51 ^a	12.81 ± .37 ^b
Calories ⁶ (kcal/100g)	81.43	76.26

¹ Means and standard deviations for 4 replications

² Means and standard deviations for 3 replications

³ Value not measured directly. Calculated as difference based on proximate analysis

⁴ CF = Control formulation

⁵ CRF = Cholesterol reduced formulation

⁶ Value based on two replications of proximate analysis. Calculated by multiplying the calories associated per gram of fat (9), protein (4), and carbohydrates (4) and adding these values.

^{a, b} Means within a row with different letters are significantly different ($p \leq 0.05$)

whole milk and fresh eggs contains 4.5 g of protein / 100 g. The protein values reported by Wu et al. (1996) are similar to the protein content found in the milk and egg mixture of this study (Table 8). The protein quality supplied by milk and egg proteins is very valuable to infants and children during growth and to the elderly who are at risk for consuming inadequate amounts of high quality protein.

Lactose and Galactose Concentrations. The concentration of lactose in the formulations without β -galactosidase treatments was not different. The addition of β -galactosidase to each formulation caused a significant decrease ($p < 0.05$) in lactose and an increase in galactose as expected (Table 9). Lactose is a disaccharide found in milk and

Table 3.9. Interaction means¹ \pm standard deviations for lactose and galactose of sweetened milk and egg mixture.

Formulations	Constituent	
	Lactose (g/L)	Galactose (g/L)
CF ² - NLR ³	28.23 ^a \pm 4.89	14.85 ^a \pm 2.57
CF - LR ⁴	3.99 ^b \pm 2.13	2.10 ^b \pm 1.13
CRF ⁵ - NLR	31.46 ^a \pm 4.15	16.55 ^a \pm 2.19
CRF - LR	2.63 ^b \pm 2.37	1.38 ^b \pm 1.25

¹ Means and standard deviations for 3 replications

² CF = Control formulation

³ NLR = No lactose reduction

⁴ LR = Lactose reduction

⁵ CRF = Cholesterol reduced formulation

^{a, b} Means within a column with different letters are significantly different ($p \leq 0.05$)

milk products. The addition of β -galactosidase hydrolyzes lactose into its monosaccharide components, glucose and galactose, in equimolar concentrations. Addition of β -galactosidase to the formulations increased the glucose and galactose concentrations in the LR formulations. Glucose and galactose are more digestible by the body (Holsinger and Kligerman, 1991). The assay used measures galactose directly, and because lactose hydrolyzes to produce equimolar concentration of each monosaccharide, the concentration of glucose is inferred at an equal concentration. There was an average 89% lactose reduction between the CF and CRF with β -galactosidase addition.

Lipid Profile. The type of egg played a major role in the difference in the type and proportion of fatty acids found in each formulations (Table 10; Table 11). There was a 20% reduction of saturated fatty acids in the CRF formulated with the cholesterol and fat reduced egg yolk. There was also a 39.4% reduction ($p < 0.05$) in the monounsaturated fatty acids of the CRF. The amount of polyunsaturated fatty acids found in the CRF was increased by 62%. Cholesterol was significantly lowered to 44% in the CRF formulation. A 100 g portion of custard mix has 56 mg of cholesterol as listed in food value portions (Pennington and Church, 1985). Wu et al. (1996) reported that custard mix made with whole milk and fresh eggs contains 115 mg of cholesterol / 100 g.

Eggs are abundant in cholesterol (Christian and Greger, 1994). The egg yolk used in the CRF were processed to remove the cholesterol. The method involved the use of vegetable oils which are mainly composed of monounsaturated and polyunsaturated fats. The cholesterol from the yolk was solubilized into the vegetable oil which was subsequently removed from the fluid egg yolk. Some yolk lipid were also removed with the vegetable oil lipids. Some residual vegetable lipids may have remained in the yolk, contributing to the increase in polyunsaturated fatty acids (PUFA). Milk played a very minor role in the fatty acid profile and cholesterol concentration because skim milk used in both CF and CRF contains only a trace amount of fat.

Table 3.10. Means¹ ± standard deviations for fatty acids in sweetened milk and egg mixture.

Fatty acid (%)	Formulations	
	CF ²	CRF ³
Butyric - C4	0	0
Caproic - C6	0.43 ^a ± 0.31	0.62 ^a ± 0.51
Caprylic - C8	0.34 ^a ± 0.39	0.10 ^a ± 0.20
Capric - C10	0.46 ^a ± 0.42	0.38 ^a ± 0.27
Lauric - C12	0.60 ^a ± 0.26	0.10 ^b ± 0.20
Myristic - C14	0.87 ^a ± 0.09	0.72 ^b ± 0.07
Palmitic - C16	26.88 ^a ± 0.69	20.38 ^b ± 0.36
Palmitoleic - C16:1	3.71 ^a ± 0.26	1.24 ^b ± 0.14
Stearic - C18	8.88 ^a ± 0.80	8.35 ^a ± 0.29
Oleic - C18:1	41.93 ^a ± 2.13	26.44 ^b ± 0.75
Linoleic - C18:2	13.54 ^a ± 0.31	35.63 ^b ± 1.07
Linolenic - C18:3	0.70 ^a ± 0.09	4.19 ^b ± 0.23
Arachidonic - C20:4	1.67 ^a ± 0.73	1.85 ^a ± 0.18

¹ Means and standard deviations for 4 replications

² CF = Control formulation

³ CRF = Cholesterol reduced formulation

^{a, b} Means within a row with different letters are significantly different ($p \leq 0.05$)

Table 3.11. Means¹ ± standard deviations for lipid profile of sweetened milk and egg mixture.

Constituent	Formulations	
	CF ²	CRF ³
Saturated fat (%)	38.45 ^a ± 1.31	30.65 ^b ± 0.47
Monounsaturated fat (%)	45.64 ^a ± 2.04	27.68 ^b ± 0.84
Polyunsaturated fat (%)	15.91 ^a ± 1.01	41.67 ^b ± 1.16
Cholesterol (mg/ 100g)	37.20 ^a ± 11.96	20.84 ^b ± 6.90

¹ Means and standard deviations for 4 replications

² CF = Control formulation

³ CRF = Cholesterol reduced formulation

^{a, b} Means within a row with different letters are significantly different ($p \leq 0.05$)

Water Activity. The water activity of the sweetened milk and egg mix and the baked gel utilizing the mix was measured. There was no significant difference in the mix or gel water activity between the formulations ($p > 0.05$). The mix and baked gels had a mean water activity of $.95 \pm 0.01$. A study by Sutton et al. (1995) reported a similar water of 0.95 for baked custards made with lactose reduced milk and whole eggs. Water activity, the unbound water available for metabolism, is associated with the growth of microorganisms in food (Defigueiredo, 1976). Water activities in a range from .90 to .99 support the growth of a wide range of bacteria, yeast, and mold. Water activity can be lowered by the addition of solutes such as sucrose and sodium chloride. The water activity of this milk and egg mixture is at a level that indeed supports the growth of bacteria in food. The hydrolysis of lactose into its monosaccharides increased the number of molecules available to bind water. However, the increased number of monosaccharides and the level of sugar and other solutes in the mixture was not at a level to inhibit

microbial growth. Thus, the thermal processing conditions must be adequate to destroy the microbial load and ensure safety and an adequate shelf life of the product.

4. Physical and Functional Characteristics of Sweetened Formulations

Color. Hunter “L”, “a”, and “b” values were measured for the mix and top and bottom surfaces of the gel. In this color system, “L” illustrates dark to light (0 to 100), “a” illustrates red (+) to green (-), and “b” represents yellow (+) to blue (-). The type of egg was the main effect contributing to the difference in color between the formulations. There were significant differences in the “L”, “a”, and “b” values of the mixes for the four different formulations ($p < .05$) (Table 12).

The “L” and “a” values of the mix formulations were relatively close such that differences would probably have limited significance with respect to visual observations. The Hunter “b” values were lower in the CRF, as compared to the CF, and might contribute to a visual difference. The CF were more yellow than the CRF based on visual observations by the researcher. Eggs contain carotenoid pigments which may affect the egg color. The egg yolk contains xanthophylls which are fat-soluble pigments (Penfield and Campbell, 1990).

These fat soluble pigments were removed with the cholesterol during the cholesterol reduction method used to reduce the cholesterol in the egg yolks, therefore, contributing to the less yellow appearance of the CRF.

No differences in the “L” values were observed for the top and bottom surfaces of the baked gels. The “L” values for the top surface of the baked gels were similar to the “L” value of (86.5 + 1.30) for a baked custard made with whole eggs and 1% milk (Sutton et al., 1995). However, Wu (1996) reported higher “L” values (90.71 + 0.36) for the top surface of a baked custard made with whole milk and whole eggs. There were significant color differences in the “a” and “b” values between the CF and CRF of the top and bottom gels. Hunter “b” values indicated CF were significantly more yellow than the CRF

Gel Strength and Syneresis. There was no significant difference in the gel strength between the baked gels utilizing the milk and egg mix (Table 12). The gel

Table 3.12. Interaction means¹ ± standard deviations for physical measurements of sweetened milk and egg mixture.

Properties	Formulations			
	CF ² -NLR ³	CF-LR ⁴	CRF ⁵ -NLR	CRF-LR
Gel strength (g force)	17.00 ± 1.79	17.25 ± 1.60	17.00 ± 1.41	16.25 ± 1.40
Syneresis (mL)*	0.23 ± 0.48	0.10 ± 0.24	0.22 ± 0.35	0.77 ± 0.26
Viscosity (cps)*	1363 ± 94	-----	349 ± 198	-----
Color				
Top surface				
Hunter “L”	85.48 ± 1.47	85.58 ± 1.57	86.37 ± 1.49	87.11 ± 1.30
Hunter “a”*	-7.62 ± 0.34	-7.60 ± 0.32	-6.39 ± 0.25	-6.38 ± 0.19
Hunter “b”*	25.32 ± 2.66	25.16 ± 3.35	16.23 ± 1.63	16.52 ± 1.21
Bottom surface				
Hunter “L”	86.85 ± 0.92	86.63 ± 0.93	84.66 ± 0.79	86.67 ± 0.76
Hunter “a”*	-7.34 ± 0.27	-7.39 ± 0.17	-6.05 ± 0.27	-6.31 ± 0.21
Hunter “b”*	20.16 ± 0.99	19.49 ± 0.81	13.16 ± 1.03	11.78 ± 1.43
Mix				
Hunter “L”*	72.15 ± 0.29	72.19 ± 0.26	72.79 ± 0.37	72.72 ± 0.37
Hunter “a”*	-5.05 ± 0.31	-4.92 ± 0.33	-4.46 ± 0.24	-4.25 ± 0.27
Hunter “b”*	17.01 ± 0.48	17.24 ± 0.23	11.15 ± 0.61	11.30 ± 0.33

¹ Means and standard deviations for 3 replications

² CF = Control formulation

³ NLR = No lactose reduction

⁴ LR = Lactose reduction

⁵ CRF = Cholesterol reduced formulation

* Significant differences at (p ≤ 0.05)

strength of the CRF-LR baked gel was similar to the gel strength (16.33 ± 2.52 g force) of a traditional baked custard made with whole milk and whole eggs (Wu, 1996). The irreversible heat coagulation of the proteins in some gel structures bind the food materials together so firmly (over coagulation) that it expels the liquid out (Yang and Baldwin, 1995). Syneresis, the drainage of a liquid from a gel, is an indication of gel strength. Since the gel strength did not change between the formulations, there should have been no significant difference in syneresis between formulations. Therefore, one can infer that the method used to measure syneresis was not adequate. The CRF with the lactose reduction had a higher degree of syneresis. Gel strength is attributed to the structure of the protein gel. The milk and eggs provide proteins which contribute to the gel formation. In addition, the milk provides the mineral salts for the coagulation of the egg proteins, which are the major contributors of the gel structure (McWilliams, 1993).

Viscosity. There was a significant difference in the viscosity of the CF and CRF ($p < .05$) (Table 12). The CF was more viscous than the CRF formulation. Egg yolk contains lecithin, a phospholipid, which acts as an emulsifier (Nawar, 1985). Lecithin promotes oil- in-water emulsions. The process used to remove the cholesterol from the cholesterol reduced egg yolk seems to have removed the lecithin from cholesterol reduced egg yolk. Consequently, the lecithin which acts as a thickening agent is present in lower amounts in the CRF than in the CF causing a less viscous CRF mix.

5. Chemical Composition of Unsweetened Mix

Proximate Analysis. The principle difference in formulation between the unsweetened and sweetened formulations was the absence of sucrose in the unsweetened formulation (Table 13). Although the two formulation were not statistically compared it is valuable to note the reduction in carbohydrates and calories in the unsweetened mix formulations as compared to the sweetened mix (Table 8; Table 13). The unsweetened CRF was lower in fat than the CF (Table 13).

Lactose and Galactose Concentrations. Concentration of lactose was slightly higher in the unhydrolyzed unsweetened formulation as compared to the unhydrolyzed

Table 3.13. Means¹ ± standard deviations for proximate analysis of unsweetened milk and egg mixture.

Constituent (g/ 100g)	Formulation	
	CF ³	CFR ⁴
Fat	0.63 ^a ± 0.071	0.45 ^b ± 0.021
Protein	5.43 ^a ± 0.11	5.36 ^a ± 0.10
Moisture	87.11 ^a ± 0.37	87.66 ^a ± 0.10
Ash	1.00 ^a ± 0.010	1.03 ^a ± 0.035
Carbohydrates ²	5.83 ^a ± 0.51	5.51 ^a ± 0.07
Calories (kcal/ 100g) ⁵	50.71	47.53

¹ Means and standard deviations for 3 replications

² Value not measured directly. Calculated as difference based on proximate analysis

³CF = Control formulation

⁴ CFR = Cholesterol reduced formulation

⁵Value based on proximate analysis of three replications

^{a, b} Means within a row with different letters are significantly different ($p \leq 0.05$)

sweetened formulations. This could be attributed to the absence of sucrose in the formulation. Therefore, a 1 g aliquot of the unsweetened mix would have more skim milk proportionally than a 1 g aliquot of the sweetened mix. There was an average 94% lactose reduction between the CF and CRF with the β -galactosidase addition (Table 14).

Lipid Profile. The relationships of fatty acids and the lipid profile were similar in the unsweetened mix (Tables 15,16) as were found in the sweetened mix (Tables 10,11). Some short chain fatty acids were not recovered in the unsweetened mix method as compared to the sweetened mix (Tables 15; Table 10, respectively). This result may be attributed to the fact that we are working with such a small amount of lipid, less than 1 g. There was a 16% reduction of saturated fatty acids in the CRF that used the cholesterol and fat reduced egg yolk. There was also a 42% reduction ($p < 0.05$) in the monounsaturated fatty acids of the CRF. The amount of polyunsaturated fatty acids found in the CRF increased by 63% (Table 16). The cholesterol in the CRF was significantly lowered by 37%.

Water Activity. The water activity of the unsweetened milk and egg mix and the baked gels utilizing the mix was measured. There was no significant difference in the mix or gel water activity between the formulations ($p > 0.05$). The mix and gel had a mean water activity of $.96 \pm 0.01$.

6. Physical and Functional Characteristics of Unsweetened Formulations

The physical and functional characteristics of the unsweetened mix (Table 17) were similar to the characteristics in the sweetened mix (Table 12). The Hunter “b” values were comparably higher in the unsweetened mix, probably because there was less dilution of the egg yolk without the addition of sugar. This result might mean the unsweetened mix would be visibly more yellow than the sweetened mix but this was not determined. The relationships among the color values were very similar in the unsweetened mix to those reported for the sweetened mix (Table 17, Table 12, respectively). The gel strength of the unsweetened mixes were comparable to those of the sweetened mixes. The unsweetened

Table 3.14. Interaction means¹ ± standard deviations for lactose and galactose of unsweetened milk and egg mixture.

Formulations	Constituent	
	Lactose (g/L)	Galactose (g/L)
CF ² - NLR ³	37.91 ^a ± 3.21	19.94 ^a ± 1.69
CF - LR ⁴	3.47 ^b ± 2.93	1.82 ^b ± 1.54
CRF ⁵ - NLR	39.56 ^a ± 4.14	20.81 ^a ± 2.17
CRF - LR	1.06 ^b ± 1.02	0.55 ^b ± 0.54

¹ Means and standard deviations for 3 replications

² CF = Control formulation

³ NLR = No lactose reduction

⁴ LR = Lactose reduction

⁵ CRF = Cholesterol reduced formulation

^{a, b} Means within a column with different letters are significantly different ($p \leq 0.05$)

Table 3.15. Means¹ ± standard deviations for fatty acids in unsweetened milk and egg mixture.

Fatty acid (%)	Formulation	
	CF ²	CRF ³
Butyric - C4	0 ^a	0 ^a
Caproic - C6	0.67 ^a ± 0.03	0.76 ^a ± 0.17
Caprylic - C8	0 ^a	0.15 ^a ± 0.27
Capric - C10	0 ^a	0.65 ^a ± 0.09
Lauric - C12	0 ^a	0 ^a
Myristic - C14	0.75 ^a ± 0.26	0.87 ^a ± 0.33
Palmitic - C16	26.63 ^a ± 0.98	20.11 ^b ± 0.98
Palmitoleic - C16:1	3.51 ^a ± 0.07	1.26 ^b ± 0.13
Stearic - C18	8.84 ^a ± 0.21	8.45 ^a ± 0.23
Oleic - C18:1	44.34 ^a ± 0.85	26.48 ^b ± 0.33
Linoleic - C18:2	13.06 ^a ± 0.41	35.09 ^b ± 1.49
Linolenic - C18:3	0.60 ^a ± 0.05	4.15 ^b ± 0.22
Arachidonic - C20:4	1.58 ^a ± 0.08	2.03 ^b ± 0.11

¹ Means and standard deviations for 3 replications

² CF = Control formulation

³ CRF = Cholesterol reduced formulation

^{a, b} Means within a row with different letters are significantly different ($p \leq 0.05$)

Table 3.16. Means ¹ ± standard deviations for lipid profile of unsweetened milk and egg mixture.

Constituent	Formulation	
	CF ²	CRF ³
Saturated fat (%)	36.90 ^a ± 1.21	30.99 ^b ± 1.79
Monounsaturated fat (%)	47.86 ^a ± 0.80	27.74 ^b ± 0.44
Polyunsaturated fat (%)	15.25 ^a ± 0.46	41.27 ^b ± 1.79
Cholesterol (mg/ 100g)	24.87 ^a ± 4.47	15.78 ^b ± 0.85

¹ Means and standard deviations for 3 replications

² CF = Control formulation

³ CRF = Cholesterol reduced formulation

^{a, b} Means within a row with different letters are significantly different ($p \leq 0.05$)

Table 3.17. Interaction means $^1 \pm$ standard deviations for physical measurements of unsweetened milk and egg mixture.

Properties	Formulation			
	CF ² -NLR ³	CF-LR ⁴	CRF ⁵ -NLR	CRF-LR
Gel strength (g force)	17.67 \pm 1.21	17.83 \pm 1.47	17.67 \pm 3.06	16.42 \pm 1.59
Syneresis (mL)*	0.02 \pm 0.04	0.02 \pm 0.04	0.63 \pm 0.59	0.27 \pm 0.27
Viscosity (cps)*	1478 \pm 244	-----	653 \pm 342	-----
Color				
Top surface				
Hunter "L"*	86.97 \pm 1.40	86.92 \pm 0.89	88.39 \pm 0.73	88.26 \pm 1.22
Hunter "a"*	-10.19 \pm 0.93	- 10.52 \pm 0.83	- 8.02 \pm 0.26	-8.04 \pm 0.15
Hunter "b"*	32.18 \pm 2.39	32.42 \pm 3.08	19.18 \pm 1.37	19.16 \pm 1.62
Bottom surface				
Hunter "L"*	88.05 \pm 1.23	88.61 \pm 0.91	89.59 \pm 1.04	89.03 \pm 0.73
Hunter "a"*	- 7.98 \pm 0.27	- 7.93 \pm 0.27	-6.87 \pm 0.25	-6.90 \pm 0.34
Hunter "b"*	19.60 \pm 1.85	19.88 \pm 1.49	12.00 \pm 1.42	12.45 \pm 2.78
Mix				
Hunter "L"*	73.70 \pm 0.42	73.87 \pm 0.39	74.58 \pm 0.48	74.34 \pm 0.53
Hunter "a"*	-5.48 \pm 0.28	-5.66 \pm 0.37	-5.31 \pm 0.17	-5.04 \pm 0.32
Hunter "b"*	17.94 \pm 0.61	17.74 \pm 0.32	0.94 \pm 0.49	11.26 \pm 0.52

¹ Means and standard deviations for 3 replications

² CF = Control formulation

³ NLR = No lactose reduction

⁴ LR = Lactose reduction

⁵ CRF = Cholesterol reduced formulation

* Significant differences at ($p \leq 0.05$)

CF exhibited very low amounts of syneresis. The mix viscosities of the unsweetened mixes were higher, as compared to those of the sweetened mixes.

E. Conclusion

A nutritionally enhanced fluid milk and egg mix can be formulated to have a lower fat, cholesterol, and lactose content while still having a protein content comparable to the control product. The thermal process that was applied provided a product with a substantial margin of safety without jeopardizing the product's functional characteristics. The milk and egg mix proved to be versatile; it could be processed as a sweet mix intended for dessert applications or as an unsweetened product intended for entree type applications. This value added type of product could be very beneficial to the foodservice industry. It provides convenience by saving preparation time when preparing foods that contain milk and eggs as ingredients while presenting a nutrition profile that enhances nutrition and reduces lactose digestibility concerns.

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