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# Effects of Homogenization and Ultra-high Temperature Processing on the Properties of Whole Milk Concentrated by a Multiple-Membrane Separation System

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# EFFECTS OF HOMOGENIZATION AND ULTRA-HIGH TEMPERATURE PROCESSING ON THE PROPERTIES OF WHOLE MILK CONCENTRATED BY A MULTIPLE-MEMBRANE SEPARATION SYSTEM

by

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# ABSTRACT

Effects of Homogenization and Ultra-high Temperature Processing on the Properties of Whole Milk Concentrated by a Multiple-Membrane Separation System

by

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Three different concentrated whole milks (2.5x, 2.75x, and 3.0x) were produced by mixing equal parts of ultrafiltration retentate of whole milk and reverse osmosis retentate of the UF milk permeate. The concentrated whole milks were ultra-high temperature processed by direct steam injection (140.6°C) followed by flash cooling and two-stage homogenization pressures (2500/500 psi, 3500/700 psi, or 4500/900 psi). The milk concentrates were packaged aseptically and stored at room temperature. On the other hand, the milk concentrates produced by the RO single membrane system with the same concentration levels served as the control. Physicochemical properties of the milks were surveyed every 2 weeks during a 6-month storage period.

The milk concentrates combined from the blending of multiple-membrane retentates showed the expected concentrations of all major nutrients except nonprotein nitrogen. A 20% to 32% shortage of nonprotein nitrogen permeated through the RO membrane during the production of the concentrated whole milks. Over the 6 months' storage, nonprotein nitrogen content did not

significantly change in the 2.5x, 2.75x, and 3.0x concentrated whole milks. No microbial growth or enzyme activity was measured or observed in the samples collected. Milk concentrated 2.5x with 4500/900psi homogenization pressure did not show cream plug formation during the first 5 months of storage. Milk concentrated 2.75x with 4500/900 psi homogenization pressure had the approximate cream plug level of the 2.5x concentrated milk at 4 months of storage. Milk concentrated 3.0x with 4500/900 psi homogenization pressure showed cream plugging at 2.5 months. As higher homogenization pressure was applied to the milk concentrates, less creaming occurred at all milk concentration levels.

Homogenization at all pressures did not reduce or eliminate sedimentation during storage. The milk concentrates from the control RO membrane processing showed less sedimentation than did the concentrates from the multiple membrane system at the same homogenization pressure (2500/500 psi). The higher the concentration of total milk solids, the more sedimentation occurred. Viscosity was not affected by homogenization pressure in any of the concentrated whole milks.

(82 pages)

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Chien-Ti Chang

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| cps | = Centipoise |
| min | = Minutes |
| MW | = Molecular weight |
| N | = Nitrogen |
| NPN | = Nonprotein nitrogen |
| RO | = Reverse osmosis |
| TN | = Total nitrogen |
| TS | = Total solids |
| UF | = Ultrafiltration |
| UHT | = Ultra-high temperature |
| x | = Times |

# INTRODUCTION

Membrane separation technology can modify the composition of milk to produce milk concentrates with advantages to both consumers and manufacturers. Ultrafiltration (UF) and reverse osmosis (RO) applied in milk products are the most popular membrane-processing technologies. Ultrafiltration and reverse osmosis both have excellent concentrating capabilities. Ultrafiltration allows small molecules such as water-soluble vitamins, lactose, minerals, water, and nonprotein nitrogen compounds to pass through as permeate. Reverse osmosis retains all components in milk by removing only water. A UF pilot plant system can successfully concentrate whole milk to 47% total solids milk retentate, and neither fat nor proteins appear in the permeate. A 2x concentrated whole milk by RO can be ultra-high temperature (UHT) treated to obtain a milk concentrate with a long shelf life and good quality. Milk concentrates from UF are usually used for cheese making and fermented milk products. Milk concentrate from RO can be used in ice cream, yogurt, cheese, and milk powder (33).

In 1971, Glover (32) tried to develop a two-stage process on a laboratory scale to concentrate milk by UF followed by RO of the permeate with subsequent recombination of the concentrates from the two operations. The original purpose was to remove proteins from the original milk before RO processing. This idea is attractive for its potential to make considerably high concentration of whole milk by sequentially using UF and RO at ambient temperature instead of using RO alone. Therefore, possible high heat damage to concentrated whole milk traditionally produced by evaporation can be avoided. In order to use the multiple-membrane concentrated whole milk, proper UHT conditions and homogenization pressures must be used.

Therefore, in practice, UF and RO membrane systems combined with UHT technology could show tremendous potential in the dairy industry.

This research study focused on utilizing the specific characteristics of UF and RO membrane separation systems to produce 2.5x, 2.75x, and 3.0x concentrated whole milks. This system could save energy, as well as shipping and packaging costs. The concentrates contain all the original milk components such as protein, fat, vitamins, minerals, lactose, and nonprotein nitrogen compounds. After UHT processing with selected homogenization pressures, the final product should have a long shelf life and good quality.

# LITERATURE REVIEW

## Membrane Processing of Milk

*Ultrafiltration.* In some European countries, UF is used to concentrate milk before transporting it from dairy farms to dairy plants. Benefits to dairy farmers and cheese makers include reduced transport and refrigeration costs, lower rennet costs, and increased cheese yields (47). In 1977, Covacevich and Kosikowski (20) produced cream cheese from UF skim milk concentrate. Other successful examples include Maubois et al. (51), who patented a method to produce high-moisture cheeses from ultrafiltered milk. Ernstrom et al. (26) used ultrafiltration to produce a cheese base from whole milk for process cheese and process cheese food.

The principle of membrane separation is that a driving force forces a pressurized fluid through a semipermeable membrane, filtering components of the fluid according to size, charge, and shape (25, 34). The pore size of the membrane determines the pressure required, the solute retained, and the specific application. Therefore, membrane separation techniques are categorized as 1) particle filtration in which the membrane pore diameter is about 1.0 micrometer, suitable for removing dust and larger cells; 2) microfiltration in which the membrane pore diameter is between 1.0 and 0.02 micrometer, used in sterilizing cell culture media by sieving out bacteria; 3) ultrafiltration in which the membrane pore diameter is from 0.002 to 0.2 micrometer, to fractionate and separate proteins; and 4) reverse osmosis (hyperfiltration) in which the membrane pore diameter is less than 0.001 micrometer, traditionally used in desalting sea water and purifying drinking water or laboratory water (25, 57).

Polymer materials constitute filtration membranes having different characteristics based on the type of polymer. Cellulose acetate has limitations outside the pH range of 3 to 7. It is sensitive to temperatures above 35° C and is intolerant to chloride. Polyamides can be used at wider pH ranges and higher temperatures than cellulose acetate. Polysulfone membranes are dominant in industrial applications because of their resistance to temperatures up to 100° C and the pH range at which they can be used (pH 1 to 14). Furthermore, they can more effectively tolerate chlorine and have more hydrogen-bonding capabilities (25, 57).

Another important aspect related to specific membrane applications is membrane configuration. This includes tubular, flat sheet, spiral-wound, and hollow-fiber membrane types. These different configurations affect packing density, pumping energy, fouling resistance, and blocking of the flow channels, and have advantages and disadvantages in different applications (57).

In crossflow membrane filtration, the feed-flow stream is separated into two effluent flow streams: The permeate, which contains small molecules, passes through the membrane; and the retentate, which consists of large solutes and suspended solids, is retained by the membrane. When whole milk is ultrafiltered, the retentate includes concentrated protein, fat, and insoluble salts. Lactose, minerals, water soluble vitamins, and small amounts of nonprotein nitrogen pass through the membrane as permeate (29, 44, 84). The changes in concentration and chemical properties of milk constituents of retentate and permeate during UF should be understood to find further applications. Green et al. (35) chemically characterized milk concentrates from UF processing. Premaratne and Cousin (61) determined the change in concentrations of major milk components during concentration of skim milk to 5x by UF. Bastian et al.

(10) reported retention and recovery of milk components during UF from acidified and unacidified milk.

The large amount of UF permeate, which is composed mainly of lactose, is the by-product of processing UF whole milk and whey. Barbano et al. (8) suggested this UF permeate contains small amounts of specific products that could have an impact on functional or flavor properties of UF milk retentate-based products. In 1980, Cotton (19) reported the utilization of UF permeate as an animal feed, syrup and alcohol production by hydrolysis, lactic acid and antibiotics by fermentation for human food, and methane by anaerobic fermentation for industrial fuel.

*Reverse Osmosis.* Reverse osmosis is used mainly in processing waste treatment and pure water makeup. The dairy industry can use RO to reduce milk transportation costs (21). Reverse osmosis concentrates from whole milk can be used to manufacture liquid milk products, butter, skim milk powder, and yogurt (23, 33). Reverse osmosis is similar to UF but uses operating pressures between 20 and 100 bar. The membranes possess a closely knit structure (approximately 5 to 30 nanometer) (50). The principle of RO is the chemical potential between two sides--the solvent and solution achieves equilibrium by the osmotic pressure from the solute. When the applied pressure is greater than the osmotic pressure in the solution, the solvent (water) will inversely diffuse through the membrane into the solvent. The osmotic pressure is related to how much energy input is required and the permeate flux rate. It is proportional to the concentration of solute and inverse to molecular weight. In concentrating whole milk by RO, lactose and other minerals contribute to higher osmotic pressure than proteins and fat (25, 33, 34). This unique aspect of concentrating ability removes pure water from the raw product without damaging the final

product and saves energy. For example, evaporation with three or four effects requires 126 to 180 kWh to remove one metric ton of water. In RO systems, concentrating milk needs 9 to 19 kWh to remove the same amount of water. Furthermore, an evaporator occupies a larger space compared with a more compact RO system (50).

In concentrating by RO, only water is theoretically removed from the milk. The RO retentate contains the original milk components in less water. During RO concentration the permeate flux declines with increasing solids content, and the process becomes uneconomical at concentration levels above 25-30% total solids under most practical operating conditions (45, 50). This RO concentration level is confined to 2x volume reduction. The reason is that the osmotic pressure increases in the concentrate due to the accumulation of proteins at the membrane surface in the presence of the milk salts. This change reduces the rate of water permeation and the effective driving force (50).

Ultrafiltration has an excellent performance in concentration of whole milk and can result in six-fold concentration of product. However, this concentrated milk does not contain the full complement of lactose and minerals as found in concentrated milk produced by other processes such as evaporation or reverse osmosis. Also, high bacterial count and somatic cells, as well as high fat and protein concentration, can lead to reduced ultrafiltration flux (9).

In Australia, Kocak (42) investigated the stability of RO concentrated milk or diluted RO concentrated milk for UHT processing in order to use it as a raw material for dairy products manufacture. The benefit is that RO/UHT milk can target foreign markets because of the transportation benefits of reduced volume, long shelf life without refrigeration, and desirable qualities.

**UHT Processing of Milk**

The aim of UHT processing is to obtain a long shelf-life product without refrigeration. UHT treatment is defined as heating a continuous flow of product at not less than 137°C for at least two seconds followed by packaging under aseptic conditions (15, 16). There are two heating methods--direct and indirect heating. In indirect heating, energy is transferred between the product and the heating source by a heat exchanger without physical contact. In direct heating, the product directly contacts the heating source by either injection or infusion. Obviously, the direct heating system more easily obtains the high rates of heating and cooling than the indirect heating system. The direct heating system has higher capital and energy costs than indirect heating due to a more complex design and low regeneration efficiencies (16). For highly viscous products, a direct heating system may provide a safe and convenient way to UHT process. In 1969, Zadow (83) compared direct and indirect UHT treatment of milk, and found that the indirect treatment was more severe than direct in ferricyanide-reducing value (that may indicate browning reaction), product color, and whey protein denaturation. In 1984 Ramsey and Swartzel (62) reported the direct system tended to produce more sediment and less fat separation than indirect.

Under such high temperature, the physicochemical, microbiological, and nutritional properties of milk can result in certain changes. The purpose of UHT treatment is to destroy all vegetative cells, but some spores may survive. The changes of physicochemical properties of milk, which include color, flavor, sedimentation, separation, nutritional value, and gelation, may be related to the heat stable enzymes (53).

*Age Gelation.* Age gelation is a detrimental defect of UHT milk. The age gelling of UHT milk can occur by a multiple-factor reaction. Many researchers have tried to explain this important phenomenon during storage of UHT milk (1, 2, 46, 65). There are two processes involved in age thickening. One is a nonenzymatic physicochemical process and the second is an enzymatic proteolytic process. The former process includes destabilization of casein micelle structure and its salt balance system, and complexing of denatured whey protein and casein; Maillard reactions lead to formation of covalently bonded polymers, pH change, and the interaction of casein and carbohydrate.

The second process is mainly a proteolytic enzyme reaction by heat-stable proteinases from the raw milk itself or from psychrotrophic bacteria (48). The three-dimensional network from the interaction of casein micelles will trap fat globules and whey proteins. From electron microscope studies, UHT milk possesses a considerable amount of small-sized casein micelles with rough surfaces. During storage, and before the gelation occurs, the size of casein micelles becomes larger, which may signal the start of gelation (17, 77).

There are some methods to prevent age gelation. Using additives such as polyphosphates, manganese (II) salts, polyhydric alcohols, and phosphatides can delay gelation (77). The proper preheating conditions, adjusting the pH of raw milk to 7.4, prolonging the holding time, homogenization after sterilization, and a lower storage temperature also improve the stability of the products (53).

*Sedimentation.* Sedimentation is a potential problem in UHT milk that was early recognized by Ashton (5) and Burton (14). It is not as severe in unconcentrated milk, and aggregation or sedimentation by gravity will easily break up by agitation or stirring (22, 36). Sedimentation can be caused by certain processing conditions, such as higher sterilization temperature, using a

direct heating system, higher homogenization pressure, and higher storage temperature (58, 62). Some researchers have obtained different results about the processing conditions that affect sedimentation (15, 54, 66, 82). Wilson et al. (82) reported that more sediment occurred at 4.4°C storage temperature than at 21.2°C or 37.8°C. Samuelsson and Holm (66) found that indirect heating caused more sedimentation than direct heating.

Denaturation of the proteins or precipitation of the salts in milk may cause sedimentation due to high heat treatment (53). Burton (14) suggested the mechanism of sedimentation is the same as the fouling of heat exchanger surfaces by milk solids. Dalgleish (22) recently reported casein micelles have a tendency to sediment during several months of storage and to produce a small layer of sedimented material, which could be predicted by appropriate calculations. Small increases in the molecular weight of the casein micelles may increase the sedimentation rate significantly. Because calcium balance and addition of salts change the sedimentation, adding sodium citrate, bicarbonate, or disodium phosphate inhibits sediment formation, but adding calcium will promote sedimentation (3, 53). In addition, adjusting the pH of milk above 6.6 will help prevent sedimentation (16).

*Fat Separation.* Fat separation results from insufficient distribution and size reduction of the fat globules. It can be eliminated by proper homogenization. A downstream homogenizer favors the concentrated milk or cream, which are products sensitive to heat coagulation, and efficiently reduces fat separation (43, 55). In high-fat-content products, as higher homogenization pressure is applied, the formation of sediment and protein stability was adversely affected during storage (18). If homogenization occurs after heat treatment, denatured whey proteins and casein micelles deposit on fat globule membrane and

associate with membrane components. The new fat globules have a thicker adsorbed layer that causes fat clusters, but two-stage homogenization can eliminate them (12).

*Color.* The color of raw milk is caused by the scattering of light by the fat globules, casein micelles, colloidal calcium phosphate, and to some extent by the pigments carotene and riboflavin (10). In UHT milk the size of fat globules, distribution of milk protein, and the browning reaction contribute to the appearance and color. Ultra-high temperature processing can cause milk products to be whiter than raw milk, probably because of an increase in light scattering by denaturation of whey proteins and changes in casein micelle size. Homogenization also whitens the color of milk by producing smaller fat globules. The clustering or clumping of fat will decrease the scattering of light. Storage temperature is an important factor for browning in UHT milk (40, 53).

*Flavor.* According to a United States committee on flavor nomenclature, there are four kinds of heat-induced flavors in a UHT milk: cooked or sulfurous, heated or rich, caramelized, and scorched (71). Some researchers prefer to use "stale" flavor instead of a mix of rich or heated and caramelized flavor (74, 81). Ashton (5) summarized the flavor changes in UHT milk during heating and storage into several stages. After heating, cooked flavor is dominant and can last 2 to 3 days. UHT milk is most acceptable from 5 to 12 days old; over 12 days, flat and oxidized flavors arise. Finally, stale flavor occurs with increasing storage time. The cooked flavor is the most recognized sensory aspect by consumers. It results from the oxidation of any exposed sulphydryl groups, which largely come from the volatile sulfur-bearing component, ß-lactoglobulin (68, 72). Additives can improve flavor. Potassium iodate, sodium iodate, sodium bromate, L-cystine, and 2-acetamidoethyl-2-acetamidoethane-thiolsulfonate

were proposed or patented to inhibit formation of -SH groups and thus they have reduced cooked flavor intensities (7, 27, 67). Certain processes also help reduce cooked flavor. Swaisgood (73) designed a reactor of glass beads coated with sulphydryl oxidase placed in the down-stream of a UHT holding tube. He reported that the cooked flavor can be removed. Raw milk pretreatment improves flavor by preheating at 70 to 90°C, followed by centrifuging to remove micro-organisms, and then warming the milk at 35 to 40 °C for 10 to 20 min. This processing procedure is used to improve keeping quality of UHT milk (48).

*Nutritional Value*. The nutritional value is the most important feature of UHT milk by consumers and nutritionists. Nutritional value of UHT milk means the nutrient content, the nutrient bioavailability, and the contribution made by milk to the daily intake of essential nutrients (69). Two aspects can cause nutritional value loss in UHT milk: heat processing and product storage. The destruction of vitamins and proteins in UHT milk is most noticed and discussed by many researchers. In 1987 Oamen et al. (56) reported a greater loss occurred in folic acid (12%), vitamin B12 (18%), and vitamin C (32%) during UHT processing than in vitamins B2 and B6. In general, vitamin C, vitamin B12, folic acid, and vitamin B6 are affected to some extent during heat processing; on the other hand, the fat-soluble vitamins, biotin, pantothenic acid, nicotinic acid and vitamin B2 are hardly damaged by heat treatments (30, 65, 69). Not only can high heat influence vitamin loss, but also light and oxygen can affect vitamin loss during product storage. Vitamin B2 is sensitive to light. Vitamin C will convert to heat-sensitive dehydroascorbic acid in the presence of oxygen. The oxidative breakdown of vitamin C is related to the destruction of vitamin B12, and folic acid is subject to oxidization due to loss of vitamin C (31). Loss of lysine due to Maillard reactions may decrease the nutritional value of milk as

part of the total diet (69). However, lysine in milk is relatively in excess so the biological value of milk does not markedly change (16). According to the daily intake of total diet, loss of available lysine is prevented as much as possible in order to compensate the intake of the lysine-insufficient food. Protein denaturation by heat treatment does not affect nutritional value but can increase enzymatic digestibility (63). Whey protein denaturation by the heating process can affect immunoglobulins, lactoferrin, lysozyme, and lactoperoxidase, which can decrease the anti-infection properties of milk. But there is a controversy whether these anti-infection or growth-supporting properties of "cow" milk are necessary to humans (60).

**Homogenization of Milk**

Homogenization is a basic processing step in dairy products manufacture. It essentially reduces the size of fat globules and increases the surface area and number of fat globules in milk but the total volume remains constant. Thus, milk fat is the major target of homogenization action. The structure of fat globules, as proposed by King (41), is composed of triglycerides in the central body and surrounded by a double layer phospholipid membrane that attaches with proteins or enzymes outside and is scattered with cholesterol and vitamin A inside. The stability of fat globules is determined by the properties of its membrane. Homogenization causes changes in membrane components. The size of fat globules ranges from about 1 $\mu$m to 15 $\mu$m in diameter in raw milk, but will decrease to less than 1 $\mu$m after homogenization depending on the homogenization pressure, the valve type and number, and flow rate (40).

Several theories explain the process of homogenization. The most accepted explanation is cavitation, in which vapor cavities are formed due to a sudden pressure drop as the fluid leaves the valve clearance and these quickly

collapse when the fluid passes into a region of higher pressure (38, 40). This primary main effect of homogenization alters some physical and chemical properties of milk. It can prevent creaming in fluid milk products, increase foaming capability, and give milk a whiter appearance. Cream plug formation can be reduced by homogenization because of both the less buoyant or separating force on small fat globules and retarding separation by Brownian movement and surface forces (38). Homogenization enhances foaming due to the changes of fat globule membrane structure (13). Jenness and Patton (38) proposed that foam-promoting substance is released from the native fat globules membrane to increase foaming. The coloring ability of homogenized milk or cream is used to affect the color of coffee when milk is added. In ice cream mix, homogenization affects fat stabilization and the gloss of fat, and also reduces viscosity (70). Two-stage homogenization has the advantage of breaking down the clusters which come from the reformation of small fat globules after first-stage homogenization (40).

In general, homogenization provides an adequate mixing for more uniform final products. Homogenized milk has higher viscosity than unhomogenized milk. Homogenization will lower the heat stability in concentrated milk products, which may result from the increase of casein micelle adsorption on the newly created fat globule membrane and make the products more sensitive to heat-induced aggregation (12). Although homogenization could give a degree of inhibition against lipid oxidation under the condition of excessive metallic contamination and less light exposure, homogenized milk is subject to the development of light-induced off-flavors because of the changes in the fat globule membrane (39, 80). Another application of homogenization is to reduce the microbial population by disrupting the microorganisms by cavitation shock

waves resulting from imploding gas bubbles. This high pressure homogenization provides an alternative method to avoid heat treatment damage in foods (59).

## OBJECTIVES

The objectives of this research were:

1. to produce 2.5x, 2.75x, and 3.0x concentrated whole milk by combining the whole milk retentate from ultrafiltration and the retentate from reverse osmosis of ultrafiltration permeate.

2. to ultra-high temperature process the 2.5x, 2.75x, and 3.0x concentrated whole milk retentates by direct steam injection at 140.6°C for 4 sec followed by flash cooling and aseptic packaging.

3. to homogenize the 2.5x, 2.75x, and 3.0x concentrated whole milk under two-stage homogenization pressures using total pressures of 3000, 4200, and 5400 psi with stage 2 having 20% pressure value of stage 1.

4. to measure the changes in cream plug formation, sedimentation, viscosity, and nonprotein nitrogen content of the membrane concentrated and UHT-processed 2.5x, 2.75x, and 3.0x concentrated whole milk stored at room temperature every two weeks during a 6-month period.

# MATERIALS and METHODS

## Preparation of 2.5x, 2.75x, and 3.0x Concentrated Whole Milk

*Ultrafiltration of Raw Whole Milk.* Raw whole milk was pasteurized at 62.8°C for 30 min, cooled to 48.8°C, and ultrafiltered. The UF system consisted of three Osmonics UF membranes (Osmonics Inc., Minnetonka, MN), which were connected in series on a UF module cart. The UF membranes were polysulfone type with 15 to 20 Kilodalton molecular weight cutoff. The pressure drop across the membrane during processing was maintained at 20 psi, and the processing temperature was maintained at 50 to 60°C. Ultrafiltration continued until the total solids of UF retentate reached 40.1%, 43.6%, and 47.0%. This viscous milk product was immediately cooled down to 4°C and stored. The UF permeate was collected and concentrated by RO.

*Reverse Osmosis of UF Permeate.* Ultrafiltration permeate from raw milk pasteurized and ultrafiltrated as explained above was concentrated using a RO membrane system connected in series, which consisted of two AFC membranes (APV Crepaco, Inc., Cerritos, CA). A Manton-Gaulin CGC homogenizer served as a feed pump. A high transmembrane pressure of approximately 900 psi was maintained from the beginning to the end of processing. As the total solids of UF permeate concentrated to 22.4%, 25.2% and 28.0%, RO retentate was collected in order to mix with the UF retentates, which we obtained previously. The total solids of RO retentate was determined by a hand refractometer (Leica, Buffalo, NY).

For the control sample of this multiple-membrane system, raw whole milk was directly concentrated by the RO system, and the 2.5x, 2.75x, and 3.0x concentrated whole milk was obtained by volume reduction. The

transmembrane pressure was gradually reduced from 800 psi to 500 psi by an adjustment of the back pressure valve when the concentration of the milk product increased.

The UF and RO concentrates were blended in equal parts by volume to produce 2.5x, 2.75x, and 3.0x concentrates of the original milk. Whole milk concentrated to 2.5x, 2.75x, and 3.0x by RO served as the control. The flow chart of production of 2.5x, 2.75x, and 3.0x concentrated whole milk is presented in Figure 1.

**UHT Processing of the Concentrated Whole Milk**

The concentrated whole milk was UHT-processed using direct steam injection in an Alfa-Laval Sterilab® pilot plant (Alfa-Laval, Lund, Sweden). The milk product was preheated to 76 to 80°C in plate heat exchanger number 1. Steam (pressure was 90 psi) was directly injected into the milk product to 140.6°C and held for 4 sec followed by flash evaporation in a vacuum tank to cool to 74 to 77°C. Two-stage homogenization pressures at 2500, 3500, and 4500 psi (with stage 2 approximately 20% pressure rating of stage 1) were applied to the concentrated milk products. Plate heat exchangers cooled the milk product to 38 to 43°C. In a laminar flow, hyperfiltered positive pressure chamber, the sterile milk product was aseptically collected and packaged in presterilized plastic containers (125-ml capacity, Fisher Scientific Co., Pittsburgh, PA). Milk samples were stored at room temperature for the shelf-ife study. The diagram of the direct steam injection mode of the UHT system is shown in Figure 2.

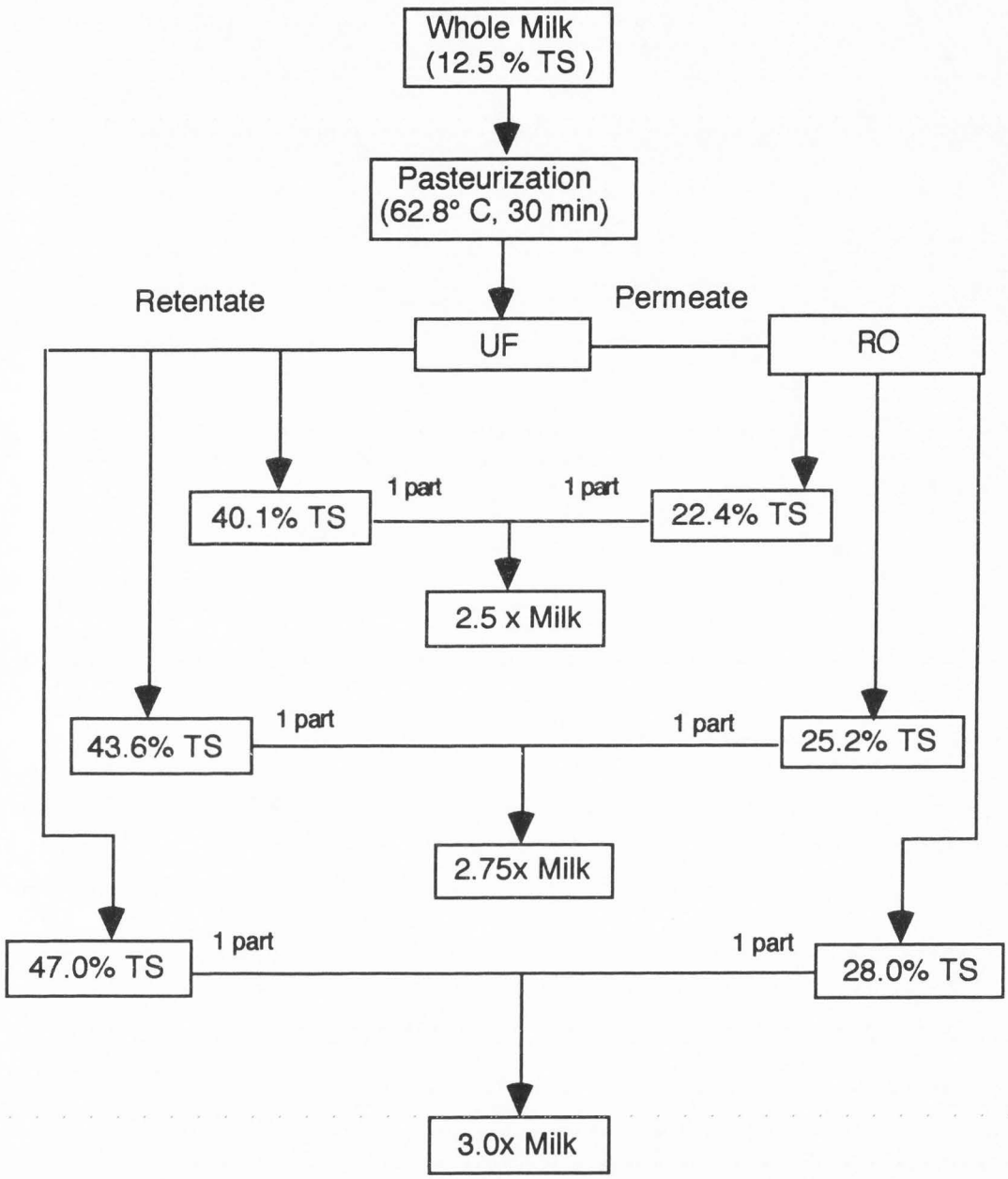


Figure 1. Flow chart of production of 2.5x, 2.75x, and 3.0x concentrated whole milk by multiple-membrane system.

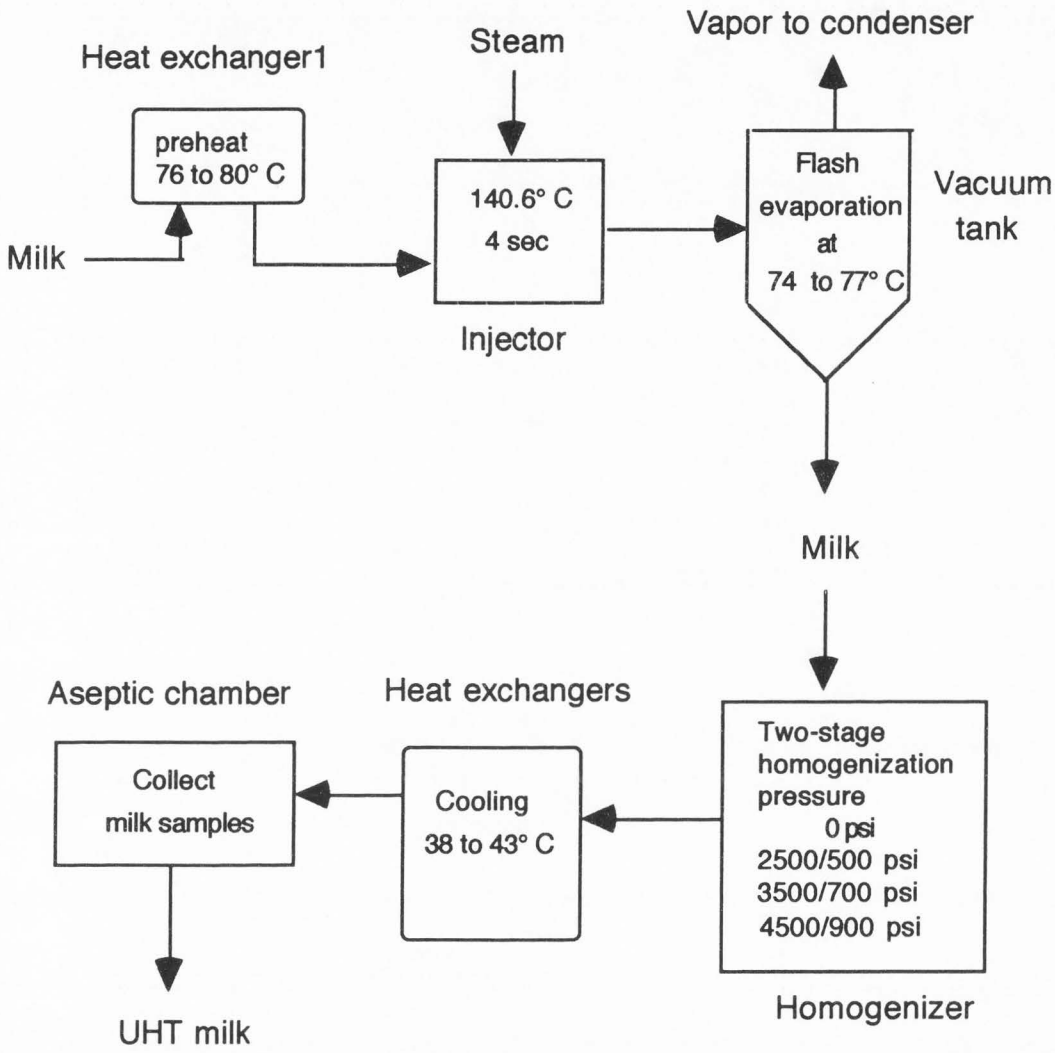


Figure 2. Schematic diagram of the direct-heating ultra-high temperature treatment in the concentrated whole milk.

**Chemical Analyses**

*Total Solids.* Total solids were measured by AVC 80 CEM microwave oven procedure (CEM Corporation, Indian Trail, NC). Operation mode 1 (100% power for 4 min) was selected using 3.0 to 4.0 g of raw milk and 2.0 to 3.0 g of RO permeate. Operation mode 2 (65% power, 10-sec interval time) was selected for 2.0 to 3.0 g of UF retentate and the blended products. Ultrafiltration permeate and RO retentate used mode 1 operation (70% power for 3 mins) for 2.0 to 3.0 g of sample size.

*Total Nitrogen.* Total nitrogen was determined by the micro-Kjeldhal method as described in the Official Methods of Analysis (960.52) of the AOAC (6). The sample size of raw milk, UF retentate, UF permeate, RO retentate, RO permeate, and blended product was 0.5 g, 0.2 g, 8.0 g, 3.0 g, 10.0 g, and 0.3 g. A Labconco Rapid Kjeldahl system (Kansas City, MO) was used. Addition of 10 ml sulfuric acid and one catalyst (Kjeltab) were put into a Kjeldhal tube with the sample and digested. After the digest cleared, it was cooled to room temperature and 15 ml distilled water was added. The distillation step was performed by gradually adding 40 ml of 50% concentrated sodium hydroxide. Steam drove off the liberated ammonia into 50 ml of 2% boric acid solution with Tashiro's indicator (0.25 g methylene blue and 0.375 g methyl red dissolved in 300 ml of 95% ethanol). Twenty-five milliliters of distillate was collected, causing a pink to grey or green color change in the boric acid solution if ammonia was liberated. During titration, the color transition stages were from green or gray to pink color. Standardized hydrochloric acid (0.02444 *N*) was used to back titrate the samples and a blank. When the first faint gray color appeared, the equivalence point was reached.

*Nonprotein Nitrogen.* Nonprotein nitrogen was measured by the micro-Kjeldahl method as described above. A 10-g sample was mixed with trichloroacetic acid at 12% (w/v) concentration followed by #42 Whatman paper filtration (64). Approximately 5.0 g filtrate of all samples was collected for digestion and nitrogen determination. Nonprotein nitrogen results were expressed as percent of milk sample weight.

*Fat.* Fat was measured by the Babcock method according to the Official Methods of Analysis (989.04 and 920.111) of the AOAC (6). The former method was used for raw milk measurement, and the latter method for UF retentate and blended measurements.

*Lactose.* Lactose content was determined by the colorimetric phenol-sulfuric acid method as described by Dubois et al. (24) and Marier and Boulet (49). All samples except RO permeate were diluted 1:1000 in distilled water. A standard curve was prepared using monohydrate lactose powder (Mallinckrodt, Inc., Paris, KY). The concentration range of the standard was 0 μg/ml to 200 μg/ml with 20 μg/ml intervals. One milliliter of 5% phenol solution and 5 ml of concentrated sulfuric acid were added to the diluted samples, mixed well, and cooled. A Beckman DU-8B spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) was used to measure optical density. At 490 nm wavelength, 0.2 nm slit width, and 5 s of dwell time, the display reading was the average of five single readings. From the standard curve, a linear equation was calculated (linear regression) to obtain the concentration of samples from the relationship between optical density value and concentration.

*Ash.* Ash was measured by a gravimetric method as described in the Official Methods of Analysis (945.46) of the AOAC (6). Modifications included 3 g of RO permeate and 1.5 g each of UF retentate, UF permeate, RO retentate, raw milk,

and the blended products, which were weighed to four decimal places in the acid-soaked and dried crucibles. Drying was performed on a hot plate rather than in a steam bath.

*Total Calcium.* Total calcium was determined using a Perkin-Elmer AAS 3100 atomic absorption spectrophotometer (Norwalker, CT). One gram of milk sample required dry ashing as described above, followed by dissolving in 5 ml of 6 *N* hydrochloric acid. After 30 to 40 min, the acid solution with sample was diluted to 100 ml using distilled water. Two and one half milliliters of this solution were transferred to the 25-ml volumetric flask and diluted by adding 5 ml of 1.0% lanthanum, to prevent interference from other ions, and 17.5 ml of distilled water.

Standard calcium solutions (4 ppm and 12 ppm) were prepared using a stock (1000ppm) calcium standard (Mallinckrodt, Inc., Paris, KY) for linear relation measurement. The wavelength and slit were set at 422.7 nm and 0.7 nm. The display reading was the average of four single readings.

*Riboflavin.* Riboflavin was determined by the fluorometric method as described in the Official Methods of Analysis (970.65) of the AOAC (6). A Gilford Fluoro IV spectrofluorometer (Ciba Corning Diagnostics Corp., Park Ridge, IL) was used to measure fluorescence in the milk products. The samples in all steps were prepared without light exposure. One gram of each sample was diluted to 10 g using distilled water and mixed with 20 ml of extraction solution (mixture of 300 ml methyl alcohol, 100 ml pyridine, 100 ml water, and 10 ml acetic acid ). The mixture was mildly agitated for 1 h and cooled. If any precipitation or undissolved particles occurred, #42 Whatman paper filtration was applied. Riboflavin (Sigma Chemical Co. St. Louis, MO ) was dissolved in 0.02 *N* acetic acid to make 100 μg/ml of standard stock solution. The 0.1 μg/ml

of freshly prepared standard solution and the sample filtrate were added to 1 ml of 0.02 *N* acetic acid before fluorescence reading. The absorption wavelength was set at 440 nm and the emission of fluorescent radiation was set at 565 nm. The actual fluorescence reading of the sample was obtained by the subtraction from the reading of the sample being hydrolyzed. The sample was added to 0.2 ml of 10% sodium hydrosulfite (dissolved in 5% sodium bicarbonate ) to be hydrolyzed. Concentration calculation was expressed as :

mg riboflavin / ml final sample solution = [( I - Q )/ ( I' - Q' )] x (0.1x 0.001 )

where I and I' are fluorescence intensities of initial sample and standard, respectively, and Q and Q' are fluorescence intensities of hydrolyzed sample and standard after adding sodium hydrosulfite, respectively.

**Physical Properties Analyses**

*Viscosity.* Viscosity was measured using a Brookfield synchro-lectric viscometer model LVT with LV spindle No. 3 (Brookfield, Stoughton, Mass.) at room temperature. All measurements were made in duplicate and directly performed in the milk sample container using 60 rpm speed for 2 min. Results were expressed in centipoise.

*Sedimentation.* Sedimentation was monitored by measuring the thickness of sediment deposited on the bottom of the containers. If the boundary of sediment layer was not clear, the sample container was given a tilt to examine. Results were expressed as percent of milk sample height in the container.

*Creaming.* Creaming was determined by measuring the height of a fat layer on top of the sample. Results were expressed as percent of milk sample height in the container.

**Statistical Analysis**

A three-factor factorial design was used to test the changes of physicochemical properties in the shelf-life study. The three factors were concentration levels, homogenization pressure levels, and storage time period. The interaction effects included concentration levels x homogenization pressures, homogenization pressures x storage time period, concentration levels x storage time period, and concentration levels x homogenization pressures x storage time period. The responses were creaming, sedimentation, viscosity, and NPN content. The effect tests and leverage plots showed the significant influence of the whole model, each effect, and the interactions. Group means comparison by Tukey-Kramer at a = 0.05 was applied to all responses of concentrated whole milks independent of storage time period and also applied to concentrated whole milks from the multiple membrane system and RO membrane system under the same homogenization pressure.

The retention of components of the concentrated whole milks by the multiple membrane system and RO membrane system from raw milk was analyzed by Tukey-Kramer with all pairs comparison at a = 0.05.

Two replicates of each test and whole experiment were performed under all experimental conditions. All statistical analyses used JMP version 2 software (SAS Institute Inc., Cary, NC).

# RESULTS AND DISCUSSION

## Membrane Processing

The three concentrated whole milks (2.5x, 2.75x, and 3.0x) were produced by the multiple-membrane system. Four fluid streams (UF retentate, UF permeate, RO retentate, and RO permeate) at the three concentration levels were analyzed for their compositions (Tables 1, 2, and 3). The distribution of milk components between UF retentate and UF permeate can determine the UF membrane performance. Total solids (TS), fat, total nitrogen (TN), ash, and total calcium content increased in UF retentate as the concentration factor increased. Total solids served as an indicator of target concentration of UF retentate. Fat was fully retained in UF retentate. No cloudy appearance in UF permeate probably indicated no large molecules such as casein micelles, whey protein, and fat globules penetrated the UF membrane. Lactose, NPN, and riboflavin did not increase in concentration because these were present in the aqueous phase and their molecular size is smaller than the UF membrane pore size, allowing them to penetrate through the membrane.

In the three concentration levels, NPN was 0.03% in both UF retentate and UF permeate. Obviously, equilibrium of NPN between UF retentate and UF permeate was achieved regardless of the increasing total solids. Total nitrogen and NPN content in UF permeate was similar (0.04% compared with 0.03%) in the three concentration levels indicating whey proteins were retained. Total nitrogen content in UF permeate was similar to previous research results (10, 33). Glover (33) found average permeate N was 0.05% from UF of whole milk when the retentate was concentrated two-fold and NPN remained constant in both UF permeate and retentate as the concentration factor reached 5x.

TABLE 1. Composition in four streams (UF retentate, UF permeate, RO retentate, and RO permeate) of the multiple-membrane system (UF and RO) in producing 2.5X concentrated whole milk.

| Component | Raw milk | | UF retentate | | UF permeate | | RO retentate | | RO permeate | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| TS (%) | 12.17 | 0.11 | 40.02 | 0.33 | 7.07 | 0.09 | 22.61 | 0.17 | 0.62 | 0.17 |
| TN ( % ) | 0.45 | 0.01 | 2.10 | 0.10 | 0.03 | 0.00 | 0.09 | 0.02 | 0.02 | 0.00 |
| NPN (%) | 0.03 | 0.00 | 0.03 | 0.00 | 0.03 | 0.00 | 0.08 | 0.01 | 0.02 | 0.00 |
| Fat (%) | 3.51 | 0.07 | 19.53 | 0.71 | NT[a] | | NT[a] | | NT[a] | |
| Lactose (%) | 4.65 | 0.12 | 3.64 | 0.13 | 5.24 | 0.14 | 18.00 | 1.70 | 0.69 | 0.15 |
| Ash (%) | 0.62 | 0.04 | 1.50 | 0.06 | 0.53 | 0.01 | 1.69 | 0.02 | 0.12 | 0.03 |
| Total calcium (mg/100 g ) | 124.37 | 4.00 | 460.75 | 19.09 | 44.71 | 2.39 | 116.01 | 8.99 | 2.69 | 1.66 |
| Riboflavin (mg/100 g ) | 0.135 | 0.035 | 0.224 | 0.037 | 0.133 | 0.007 | 0.514 | 0.053 | 0.013 | 0.011 |

[a] NT = Not tested

TABLE 2. Composition in the four streams (UF retentate, UF permeate, RO retentate, and RO permeate) of the multiple-membrane system (UF and RO) in producing 2.75x concentrated whole milk.

| Component | Raw milk | | UF retentate | | UF permeate | | RO retentate | | RO permeate | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| TS (%) | 12.18 | 0.10 | 43.54 | 0.43 | 7.01 | 0.07 | 24.94 | 0.14 | 0.78 | 0.27 |
| TN ( % ) | 0.46 | 0.00 | 2.39 | 0.04 | 0.04 | 0.00 | 0.10 | 0.01 | 0.02 | 0.00 |
| NPN (%) | 0.03 | 0.00 | 0.03 | 0.00 | 0.03 | 0.00 | 0.08 | 0.00 | 0.02 | 0.01 |
| Fat (%) | 3.53 | 0.14 | 21.90 | 0.54 | NT[a] | | NT[a] | | NT[a] | |
| Lactose (%) | 4.74 | 0.23 | 4.00 | 0.65 | 5.37 | 0.19 | 20.24 | 0.91 | 0.33 | 0.03 |
| Ash (%) | 0.65 | 0.02 | 1.62 | 0.04 | 0.53 | 0.01 | 1.85 | 0.04 | 0.05 | 0.02 |
| Total calcium (mg/100 g ) | 115.10 | 7.95 | 471.18 | 21.00 | 41.75 | 4.18 | 130.37 | 10.56 | 5.84 | 4.62 |
| Riboflavin (mg/100 g ) | 0.130 | 0.030 | 0.222 | 0.018 | 0.133 | 0.007 | 0.517 | 0.053 | 0.012 | 0.007 |

[a] NT=Not tested

TABLE 3. Composition in four streams (UF retentate, UF permeate, RO retentate, and RO permeate) of the multiple-membrane system (UF and RO) in producing 3.0x concentrated whole milk.

| Component | Raw milk | | UF retentate | | UF permeate | | RO retentate | | RO permeate | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| TS (%) | 12.22 | 0.06 | 46.14 | 0.32 | 6.56 | 0.65 | 27.50 | 0.79 | 0.45 | 0.09 |
| TN ( % ) | 0.44 | 0.01 | 2.45 | 0.08 | 0.04 | 0.01 | 0.12 | 0.01 | 0.02 | 0.00 |
| NPN (%) | 0.03 | 0.00 | 0.03 | 0.01 | 0.03 | 0.00 | 0.09 | 0.00 | 0.02 | 0.00 |
| Fat (%) | 3.51 | 0.07 | 22.90 | 0.54 | NT[a] | | NT[a] | | NT[a] | |
| Lactose (%) | 4.42 | 0.42 | 3.28 | 0.50 | 5.14 | 0.44 | 23.35 | 0.88 | 0.42 | 0.18 |
| Ash (%) | 0.58 | 0.07 | 1.70 | 0.08 | 0.43 | 0.13 | 2.15 | 0.26 | 0.06 | 0.06 |
| Total calcium (mg/100 g ) | 121.92 | 1.56 | 544.64 | 20.69 | 33.61 | 8.60 | 147.45 | 4.29 | 8.60 | 8.96 |
| Riboflavin (mg/100 g ) | 0.123 | 0.021 | 0.219 | 0.030 | 0.112 | 0.031 | 0.523 | 0.048 | 0.010 | 0.003 |

[a] NT=Not tested

Bastian et al. (10) reported average permeate N was 0.04% at 5x concentration when whole milk was concentrated by UF.

Ash is the inorganic residue from the incineration of organic materials and it can reflect partial salts content (especially metals). Milk salts include the cation group (calcium, magnesium, sodium, and potassium) and the anion group (chloride, phosphate, and citrate), which are in appreciable amounts and are important in maintaining the conformation and stability of milk proteins. Most of them associate with proteins and fat and are important to the nutritional value of milk (35, 37). Calcium plays an essential role in bone mineralization and other vital physiological processes in the human. Milk can provide abundant calcium (37). Therefore, calcium retention during membrane processing is important in the production of concentrated whole milk. Ash in UF retentate increased gradually with concentration levels as well as total calcium, but these increases did not coincide at the 3.0x concentration level. Total calcium content in UF permeate, which is in the unbound state, ranged from 33.61 mg/100 g to 44.71 mg/100 g (Tables 1, 2, and 3).

Lactose content in UF retentate at the three concentration levels was 3.64%, 4.00%, and 3.28%, while in permeate the contents were 5.24%, 5.37%, and 5.14%. This indicates higher lactose content in permeate than in retentate at the three concentration levels and an unexpected high value at the 2.75x concentration level. The different concentration of lactose content between UF permeate and retentate is because the reduced volume is from the water phase only and the high concentration factor causes a more compact retentate. This disagrees with Bastian et al. (10), who reported lactose content was higher in retentate than in permeate. We have similar results with Glover (33), who reported 4.1% and 5.1% in retentate and permeate at 3x concentration and

3.2% and 5.2% in retentate and permeate at 5x concentration. The highest concentration level had lower lactose content in retentate but lactose concentration did not change much in permeate (Tables 1, 2, and 3 ).

Riboflavin is an indispensable nutrient in milk products, providing 34.7% of the available riboflavin in the United States (52). There is concern about riboflavin retention in membrane processing of milk. In this study riboflavin was partially retained at 0.224 mg/100 g, 0.222 mg/100 g, and 0.219 mg/100 g in UF retentate and 0.133 mg/100 g, 0.133 mg/100 g, and 0.112 mg/100 g in UF permeate at the three concentration levels. There was no significant change of riboflavin content in UF retentate when the total solids concentration levels increased. Because 65% to 95% of riboflavin is present in the free form, the bound forms such as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) contribute to the amount retained (79).

The RO membrane acts as a secondary concentrating step in the multiple-membrane system. The large amount of clear, light-greenish UF permeate, which is mainly composed of lactose, minerals, water soluble vitamins, and nonprotein nitrogen compounds, was retained by RO membrane. We expected the concentration of all components of raw whole milk to increase to the desired levels in the final product. In other words, the multiple-membrane system should perfectly exclude only water. The RO permeate, however, of the three concentration levels contained lactose, nitrogen compounds, ash, total calcium, and riboflavin (Tables 1, 2 , and 3). This could be due to damages or defects in the RO membrane, causing the loss of water-soluble components. However, if this permeation of water-soluble components is trivial, the recovery of the final products will not be influenced significantly.

The compositions of the three concentrated whole milks (2.5x, 2.75x, 3.0x) from multiple-membrane processing and RO membrane control compared with the raw whole milk are presented in Figures 3, 4, and 5. Nonprotein nitrogen was approximately 20 to 25% below the expected concentration in the three milk concentrates from the multiple-membrane system. Riboflavin and the other milk components were retained in the three concentrated milks except for the 2.75x concentrated whole milk in which fat, ash, total calcium, and NPN were lower than the target concentration. Total solids were significantly different from the target value. This result was inconsistent with the retention of milk components. Therefore, the unexpected difference of total solids (TS) from the target value probably results from the sampling errors in the procedure of determining total solids. On the other hand, the milk concentrates (with the same concentration levels as MM milk) were produced by RO single membrane from whole milk serving as the control groups, which have similar results in components retention (Figures 3, 4, and 5). Nonprotein nitrogen was not completely retained in the retentate. There was 24% to 32% of NPN lost in the three milk concentrates. In 2.75x concentrated whole milk, fat, ash, and total calcium were all retained at the target level in the final product. The RO permeate from the multiple membrane system and from the RO membrane processing contained 0.016% and 0.019% NPN (Table 4). Therefore, the loss of NPN in the RO membrane is clear. According to Versteeg's suggestion (75), losses during membrane filtration are caused by permeation of small molecules or by mechanical leaks. Unsuitable cleaning practices or accidents (such as excessive pH adjustment with cellulose acetate membranes or chlorine with composite membranes) can result in membrane damage with subsequent losses. On the other hand, the loss of fat, ash, and total calcium in

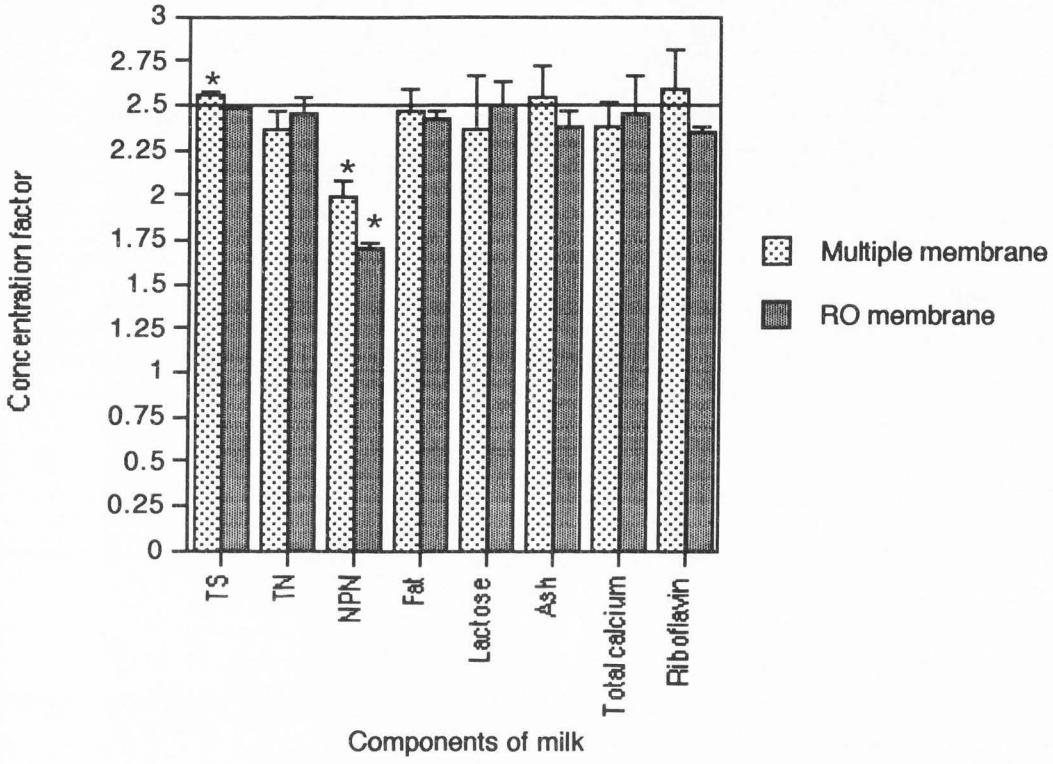


Figure 3. Retention of milk components in 2.5x concentrated whole milks produced by the multiple-membrane system and RO membrane system. Error bars are standard deviation of means. * indicates significant difference to target concentration factor (2.5x) ($P < .05$).

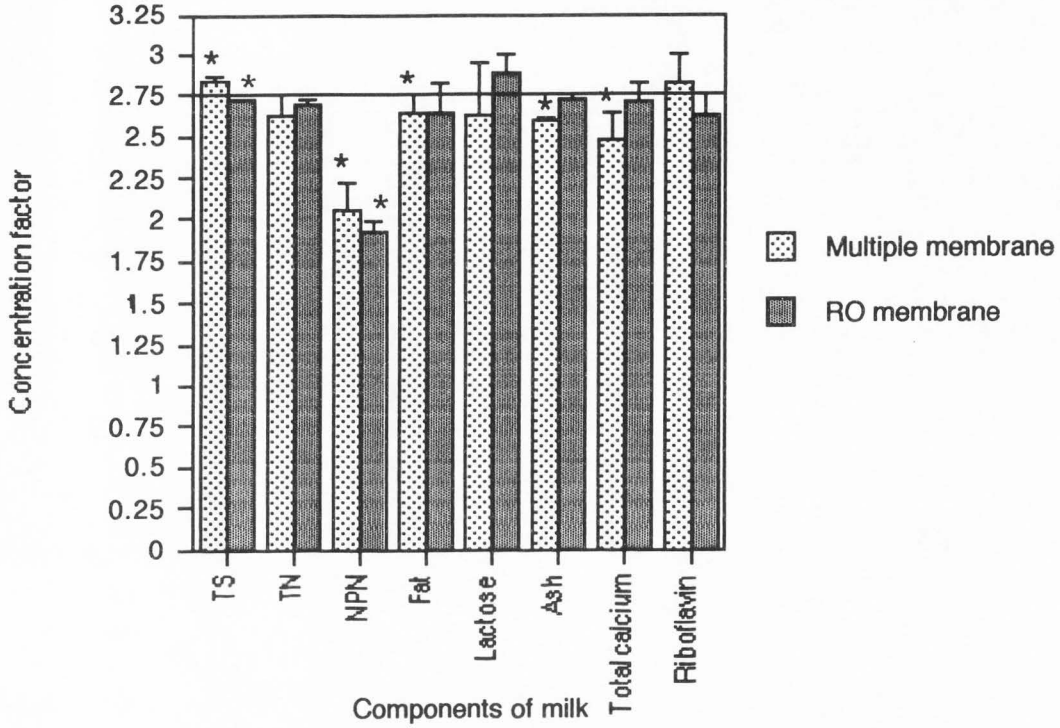


Figure 4. Retention of milk components in 2.75x concentrated whole milks produced by the multiple-membrane system and RO membrane system. Error bars are standard deviation of means. * indicates significant difference to target concentration factor (2.75x) ($P$ <.05).

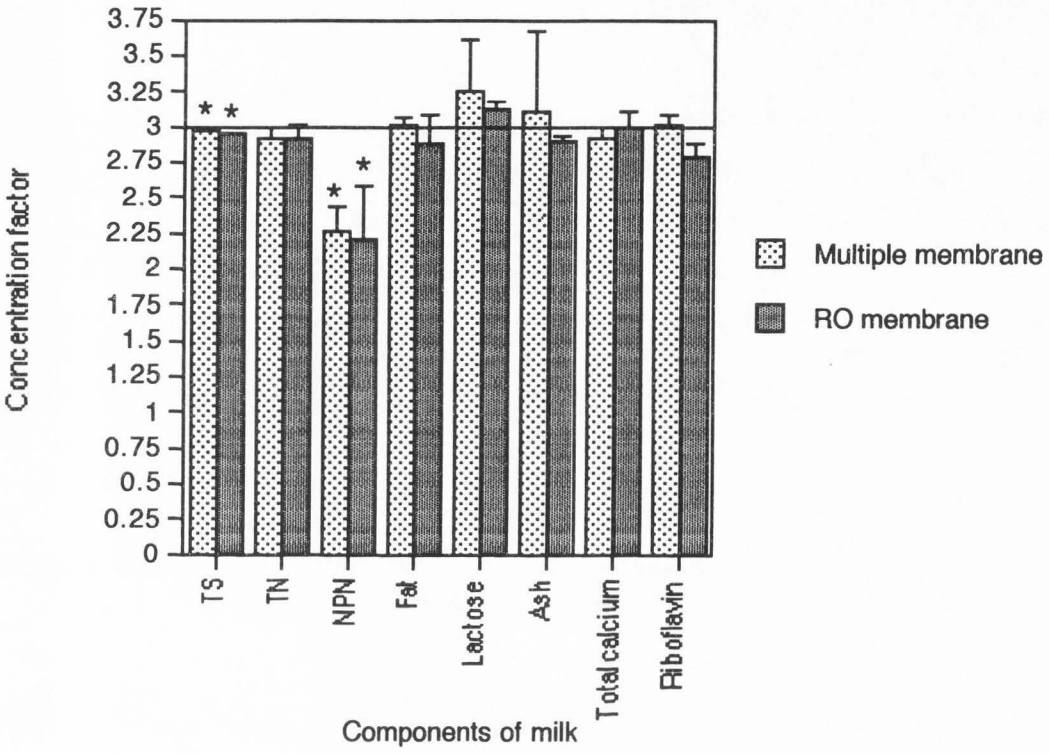


Figure 5. Retention of milk components in 3.0x concentrated whole milks produced by the multiple-membrane system and RO membrane system. Error bars are standard deviation of means. * indicates significant difference to target concentration factor (3.0x) ($P < .05$).

TABLE 4. Compositions in RO permeates from two different membrane processes (multiple-membrane system and RO single membrane) of the 2.5x, 2.75x, and 3.0x concentrated whole milk.

| Component | RO permeate | | | |
|---|---|---|---|---|
| | Multiple Membrane | | RO Membrane | |
| | Mean | SD | Mean | SD |
| TS (%) | 0.62 | 0.22 | 0.61 | 0.00 |
| TN (%) | 0.020 | 0.003 | 0.014 | 0.001 |
| NPN (%) | 0.019 | 0.005 | 0.016 | 0.002 |
| Fat (%) | NT[a] | | NT[a] | |
| Lactose (%) | 0.48 | 0.20 | 0.39 | 0.33 |
| Ash (%) | 0.08 | 0.05 | 0.06 | 0.02 |
| Total calcium (mg/100 g) | 3.594 | 3.497 | 0.594 | 0.156 |
| Riboflavin (mg/100 g) | 0.012 | 0.007 | 0.004 | 0.000 |

[a] NT=Not tested

2.75x concentrated whole milk from the multiple-membrane system (but not in the control sample) can be explained probably from the mixing step of UF retentate and RO retentate in the multiple-membrane system.

### Physicochemical Changes of UHT Concentrated Whole Milk During Storage

The physicochemical changes in UHT-concentrated whole milks during 6 months' storage were cream plug formation, sedimentation, NPN content, and viscosity. A three-factor factorial model was used for statistical analysis. The three factors were concentration levels, homogenization pressures, and storage time.

### Cream Plug Formation

The effects influencing cream plug formation are presented in Table 5. The homogenization pressure positively influenced creaming of all concentrated whole milks (Table 6). In general, at higher homogenization pressure the less

cream plug formation occurred. In the 3.0x concentrated whole milk, low homogenization pressure (2500/500psi) did not reduce cream plug formation. Increasing the concentration factor with no homogenization did not change the cream plug height. When homogenization pressures were applied, the 3.0x concentrated whole milk had the highest cream plug compared with other concentration levels. Changes of creaming with storage time of the three concentrated whole milks are shown in Figures 6, 7, and 8. The higher homogenization pressure can delay creaming longer than the lower. At medium and high homogenization pressures (4500/900psi and 3500/700psi) of the 2.5x and 2.75x concentrated whole milks, creaming started after 10 wk except that some variation existed in the 2.5x concentrated whole milk at the 8th week. The same creaming time occurred at high homogenization pressure (4500/900psi)

TABLE 5. ANOVA of a three-factor factorial design in cream plug formation in concentrated whole milk over 24 weeks' storage.

| Source | DF | MS | F ratio | Prob >F |
|---|---|---|---|---|
| Concentration level (C) | 2 | 612.265 | 108.577 | .0000 |
| Homogenization pressure (HP) | 3 | 2728.477 | 483.860 | .0000 |
| C*HP | 6 | 147.474 | 26.153 | .0000 |
| Storage time (ST) | 11 | 540.766 | 95.898 | .0000 |
| C*ST | 22 | 16.019 | 2.841 | .0000 |
| HP*ST | 33 | 37.359 | 6.625 | .0000 |
| C*HP*ST | 66 | 23.971 | 4.251 | .0000 |
| Error | 432 | 5.639 | | |

Table 6. Changes of physicochemical properties under four homogenization pressures of the 2.5x, 2.75x, and 3.0x concentrated whole milk over six months' survey.

| Homogenization pressure | Creaming (%) | Sedimentation (%) | Viscosity (cp) | NPN (%) |
|---|---|---|---|---|
| | | 2.5x conc. whole milk | | |
| No pressure | 12.88 ± 4.49$^{a}$ | 22.82 ± 10.85$^{d}$ | 38.1 ± 29.5$^{b}$ | .0608 ±.0073$^{abc}$ |
| 2500/500 psi | 7.78 ± 6.37$^{cd}$ | 18.18 ± 6.76$^{e}$ | 57.2 ± 40.7$^{b}$ | .0591 ±.0060$^{bcd}$ |
| 3500/700 psi | 5.03 ± 4.59$^{de}$ | 17.78 ± 6.10$^{e}$ | 62.1 ± 65.7$^{b}$ | .0561 ± .0061$^{de}$ |
| 4500/900 psi | 2.15 ± 3.58$^{e}$ | 19.57 ± 7.23$^{d}$ | 77.7 ± 87.4$^{b}$ | .0575 ± .0061$^{ce}$ |
| | | 2.75x conc. whole milk | | |
| No pressure | 14.15 ± 1.95$^{a}$ | 21.74 ± 7.82$^{de}$ | 121.1± 187.9$^{ab}$ | .0632 ± .0056$^{ab}$ |
| 2500/500 psi | 9.34 ± 6.17$^{cb}$ | 27.85 ± 7.66$^{b}$ | 84.5 ± 133.9$^{b}$ | .0602 ± .0056$^{abcd}$ |
| 3500/700 psi | 2.13 ± 3.38$^{e}$ | 27.28 ± 7.20$^{bc}$ | 109.9 ±193.9$^{b}$ | .0576± .0037$^{ce}$ |
| 4500/900 psi | 2.59 ± 3.51$^{e}$ | 23.15 ± 5.15$^{cd}$ | 218.0 ± 303.6$^{a}$ | .0596 ± .0075$^{abcd}$ |
| | | 3.0x conc. whole milk | | |
| No pressure | 13.08 ± 3.70$^{a}$ | 34.67 ± 3.37$^{a}$ | 100.1 ± 68.7$^{b}$ | .0632 ± .0048$^{ab}$ |
| 2500/500 psi | 11.96 ± 5.04$^{ab}$ | 34.12 ± 2.84$^{a}$ | 104.6 ± 57.8$^{b}$ | .0598 ± .0083$^{abcd}$ |
| 3500/700 psi | 8.66 ± 4.97$^{c}$ | 34.27 ± 2.64$^{a}$ | 97.1 ± 86.1$^{b}$ | .0631 ± .0078$^{ab}$ |
| 4500/900 psi | 6.61± 6.04$^{cd}$ | 36.29 ± 2.23$^{a}$ | 113.2 ± 99.2$^{b}$ | .0633 ± .0061$^{a}$ |

$^{a,b,c,d,e}$ Mean ± S.D. followed by the same superscript within the same column are not significantly different ($P > .05$)

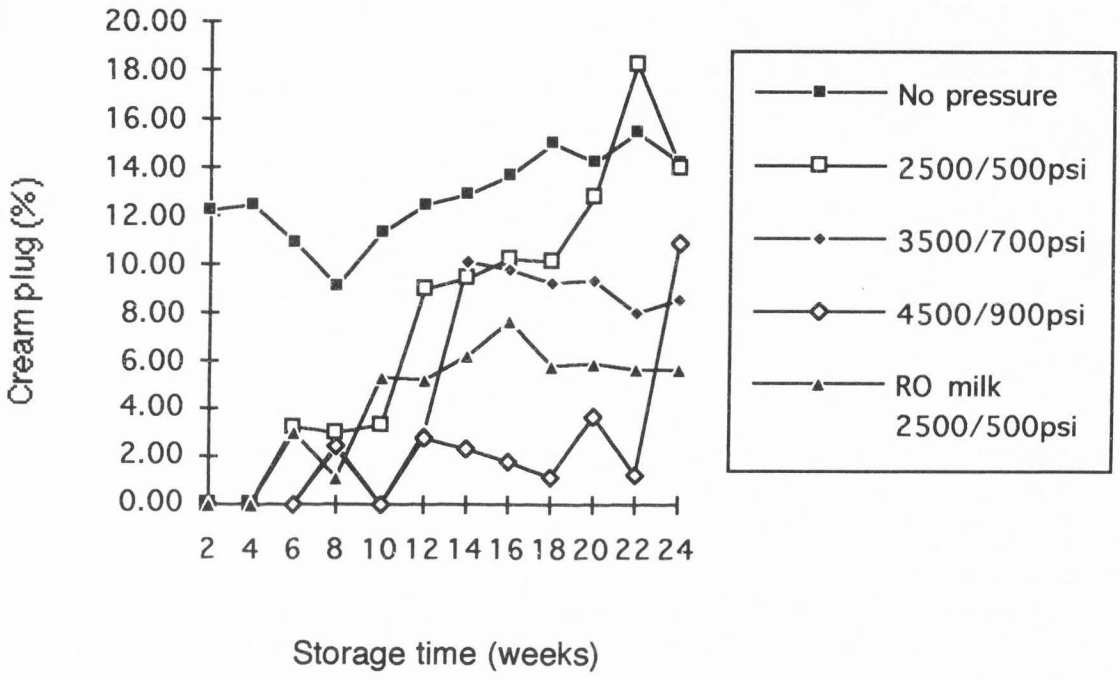


Figure 6. Changes of creaming in 2.5x concentrated whole milk with four different homogenization pressures and RO milk as the control during 24 weeks' storage.

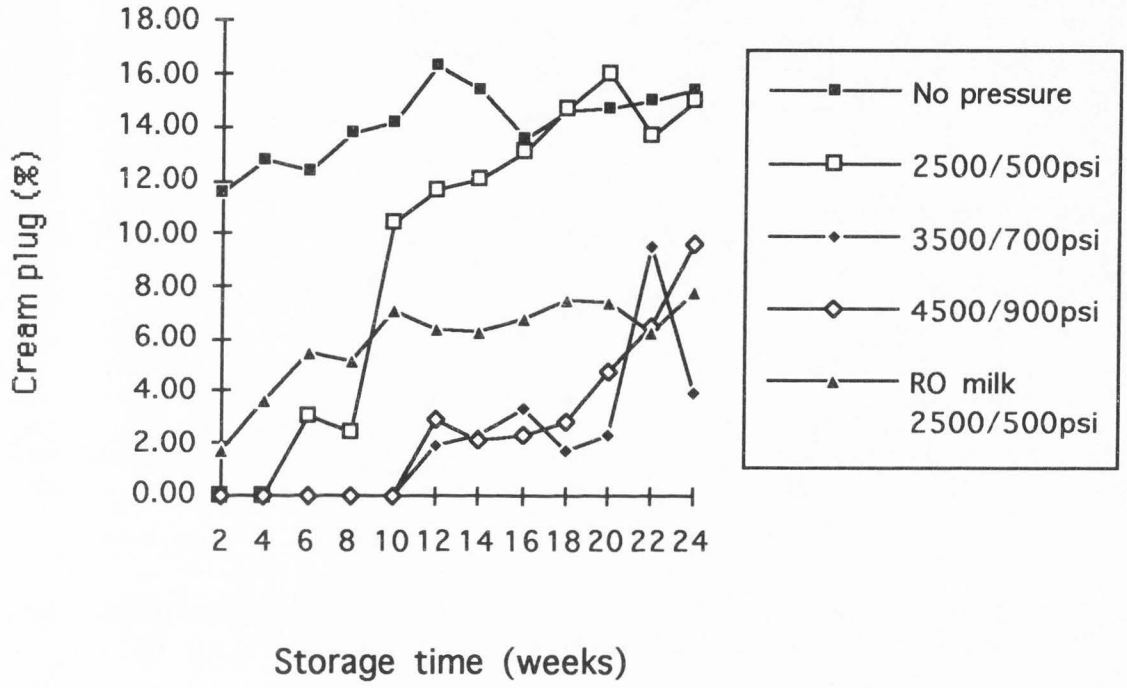


Figure 7. Changes of creaming in 2.75x concentrated whole milk with four different homogenization pressures and RO milk as the control during 24 weeks' storage.

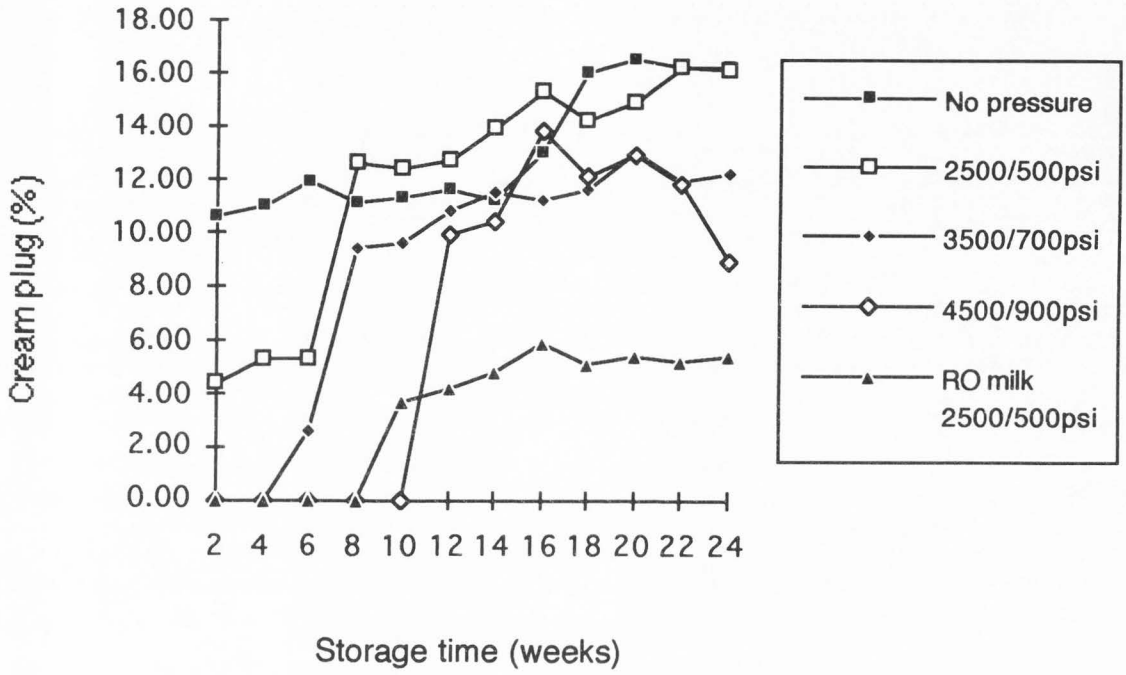


Figure 8. Changes of creaming in 3.0 x concentrated whole milk with four different homogenization pressures and RO milk as the control during 24 weeks' storage.

in the 3.0x concentrated whole milk. After the 10th wk all homogenization pressures produced similar and higher creaming effects. On the other hand, low homogenization pressure (2500/500psi) retarded creaming for 4 wk in the 2.5x and 2.75x concentrated whole milks, with less effect in the 3.0x concentrated whole milk. Comparisons of the physicochemical properties at low homogenization pressure of the RO concentrated whole milk (RO milk) and the multiple-membrane concentrated whole milk (MM milk) at three concentration levels are shown in Tables 7, 8, and 9. Creaming in the 2.5x MM milk after 12 wk was significantly higher than in RO milk. In the 2.75x MM milk after 8 wk, creaming was significantly higher than in RO milk. Creaming in the 3.0x MM milk was always higher than in RO milk during the 24 weeks' storage. The MM milk had higher creaming than the RO milk and the higher concentration level caused the higher cream plug formation. The reason for this could be that in the production of RO milk, the feed pump (a Manton-Gaulin CGC homogenizer pump) provided the appropriate flow rate and the high transmembrane pressure probably caused a homogenization action. The MM milk did not receive this extra homogenization from the MM milk system. Higher fat content in the concentrated milk is a factor to speed up cream plug formation (Figures 6, 7, and 8).

**Sedimentation**

The ANOVA test of sedimentation in the concentrated whole milk over 24 weeks' storage is presented in Table 10. In the 2.5x and 2.75x concentrated whole milks, low and medium homogenization pressures affected sedimentation inversely with decreasing sediment in the 2.5x concentrated whole milk and with increasing sediment in the 2.75x. No significant changes

TABLE 7. Changes of physicochemical properties in the 2.5x concentrated whole milk (2500/500 psi homogenization pressure) from two different membrane processings (multiple-membrane system and RO single membrane) during 24 weeks' storage.

| Membrane processing | Time (week) | Creaming (%) | Sedimentation (%) | Viscosity (cp) | NPN (%) |
|---|---|---|---|---|---|
| RO membrane | 2 | .00 ± .00$^{a}$ | 2.92 ± 4.52$^{a}$ | 44.0 ± 6.3$^{fgij}$ | .056 ± .001$^{a}$ |
| | 4 | .00 ± .00$^{a}$ | 7.45 ± 1.05$^{ab}$ | 113.5 ± 48.2$^{deh}$ | .058 ± .000$^{ac}$ |
| | 6 | 3.04 ± 3.58$^{ac}$ | 8.46 ± 2.58$^{aef}$ | 185.0 ± 39.7$^{bc}$ | .056 ± .001$^{ae}$ |
| | 8 | 1.14 ± 1.35$^{a}$ | 11.33 ± 7.33$^{bcdeghi}$ | 163.5 ± 117.0$^{bcde}$ | .057 ± .001$^{ac}$ |
| | 10 | 5.38 ± 2.53$^{bc}$ | 10.42 ± 7.89$^{ah}$ | 179.5 ± 124.2$^{bcd}$ | .056 ± .003$^{ad}$ |
| | 12 | 5.20 ± 2.49$^{bc}$ | 9.15 ± 4.94$^{ag}$ | 81.0 ± 1.4$^{efgj}$ | .059 ± .004$^{ac}$ |
| | 14 | 6.18 ± 2.53$^{bce}$ | 7.70 ± 2.50$^{ad}$ | 115.0 ± 60.8$^{cde}$ | .059 ± .002$^{ac}$ |
| | 16 | 7.63 ± 1.63$^{bf}$ | 7.68 ± 2.51$^{ac}$ | 118.0 ± 56.6$^{cdef}$ | .059 ± .002$^{ac}$ |
| | 18 | 5.78 ± 2.14$^{bce}$ | 8.28 ± 1.05$^{aef}$ | 245.0 ± 15.6$^{ab}$ | .058 ± .002$^{ac}$ |
| | 20 | 5.88 ± 1.80$^{bce}$ | 10.52 ± .21$^{ahm}$ | 329.0 ± 38.2$^{a}$ | .057 ± .002$^{ac}$ |
| | 22 | 5.63 ± 1.57$^{bce}$ | 12.11 ± 2.96$^{bcdeghij}$ | gel | .058 ± .000$^{ac}$ |
| | 24 | 5.66 ± 1.03$^{bce}$ | 12.46 ± 3.17$^{bcdeghij}$ | gel | .056 ± .000$^{ac}$ |
| Multiple membrane | 2 | .00 ± .00$^{a}$ | 9.58 ± 9.96$^{ag}$ | 26.4 ±10.9j | .056 ± .004$^{ac}$ |
| | 4 | .00 ± .00$^{a}$ | 15.49 ± 9.72$^{fghijl}$ | 35.3±14.2$^{fghij}$ | .052 ± .001$^{a}$ |
| | 6 | 3.21 ± 4.55$^{ac}$ | 19.84 ± 8.79$^{jl}$ | 31.6 ± 9.5$^{gij}$ | .058 ± .001$^{ac}$ |
| | 8 | 2.98 ± 4.21$^{ac}$ | 19.23 ± 6.85$^{jl}$ | 31.0 ± 7.4$^{ij}$ | .056 ± .001$^{ac}$ |
| | 10 | 3.33 ± 4.71$^{ac}$ | 17.83 ± 6.31$^{hl}$ | 35.2 ± 9.6$^{fgij}$ | .058 ± .001$^{ac}$ |
| | 12 | 8.96 ± 1.31$^{bg}$ | 17.61 ± 6.06$^{hl}$ | 39.4 ± 8.0$^{fgij}$ | .059 ± .006$^{ac}$ |
| | 14 | 9.48± 2.08$^{defg}$ | 18.29 ± 5.89$^{ilm}$ | 46.4 ± 20.5$^{fghij}$ | .059 ± .004$^{ac}$ |
| | 16 | 10.24 ± .75$^{dfgi}$ | 19.08 ± 5.22$^{jl}$ | 52.1 ± 9.8$^{fghij}$ | .062 ± .005$^{ac}$ |
| | 18 | 10.09 ± .79$^{dfg}$ | 17.97 ± 1.97$^{hl}$ | 63.2 ± 13.0$^{fghij}$ | .064 ± .006$^{bcde}$ |
| | 20 | 12.83 ±1.85$^{dgj}$ | 16.33 ± 2.61$^{ghijkl}$ | 74.1 ± 9.7$^{fghij}$ | .063 ± .005$^{cb}$ |
| | 22 | 18.29 ±8.00$^{h}$ | 23.66 ± 7.24$^{l}$ | 99.0 ± 48.6$^{efgi}$ | .062 ± . 004$^{cbe}$ |
| | 24 | 13.99 ± 0.41$^{ij}$ | 23.63 ± 1.83$^{l}$ | 162.2 ± 66.6$^{bcde}$ | .056 ± .014$^{a}$ |

a,b,c,d,e,f,g,h,i,j,k,l,m Means ± S.D. followed by the same superscript within the same column are not significantly different ($P > .05$)

TABLE 8. Changes of physicochemical properties in the 2.75x concentrated whole milk (2500/500 psi homogenization pressure) from two different membrane processings (multiple-membrane system and RO single membrane) during 24 weeks' storage.

| Membrane processing | Time (week) | Creaming (%) | Sedimentation (%) | Viscosity (cp) | NPN (%) |
|---|---|---|---|---|---|
| RO membrane | 2 | 2.52 ± 1.97$^{b}$ | 4.18 ± 4.87$^{a}$ | 49.0 ± 6.8$^{ab}$ | .060 ± .000$^{ac}$ |
| | 4 | 4.52 ± 1.52$^{bd}$ | 13.95 ± 2.18$^{bc}$ | 64.5 ± 4.7$^{ab}$ | .059 ± .002$^{ac}$ |
| | 6 | 5.49 ± 1.06$^{cdfe}$ | 14.08 ± 1.99$^{bc}$ | 91.0 ± 5.0$^{ab}$ | .056 ± .003$^{ab}$ |
| | 8 | 6.67 ± 3.33$^{dgh}$ | 20.34 ± 1.66$^{cd}$ | 142.5 ± 35.0$^{abcd}$ | .054 ± .001$^{a}$ |
| | 10 | 6.41± 1.92$^{dgh}$ | 10.45 ± 1.42$^{ab}$ | 179.5 ± 27.2$^{bcd}$ | .059 ± .002$^{ac}$ |
| | 12 | 5.32 ± .16$^{cde}$ | 9.68 ± 2.02$^{ab}$ | 279.5 ± 56.58$^{de}$ | .062 ± .002$^{ce}$ |
| | 14 | 7.12 ±1.57$^{eh}$ | 10.54 ± .39$^{ab}$ | 256.0 ± 13.0$^{cde}$ | .060 ± .004$^{ac}$ |
| | 16 | 6.59 ± .86$^{dgh}$ | 10.01 ± 3.17$^{ab}$ | gel | .068 ± .001$^{de}$ |
| | 18 | 8.13 ± .76$^{ghi}$ | 7.85 ± 1.76$^{ab}$ | gel | .062 ± .003$^{bcd}$ |
| | 20 | 6.69 ± 1.14$^{dgh}$ | 8.52 ± 1.12$^{ab}$ | gel | .060 ± .004$^{ac}$ |
| | 22 | 6.95 ± 1.21$^{dgh}$ | 10.43 ± .38$^{ab}$ | gel | .064 ± .002$^{ce}$ |
| | 24 | 7.84 ± .94$^{fh}$ | 10.50 ± .46$^{ab}$ | gel | .064 ± .002$^{ce}$ |
| Multiple membrane | 2 | .00 ± .00$^{a}$ | 31.93 ± 15.65$^{fg}$ | 31.5 ± 9.3$^{a}$ | .061 ± .006$^{cb}$ |
| | 4 | .00 ± .00$^{a}$ | 27.63 ± 12.03$^{dg}$ | 46.4 ± 19.3$^{ab}$ | .064 ± .005$^{ce}$ |
| | 6 | 3.03 ± 4.29$^{bc}$ | 28.43 ± 9.85$^{dg}$ | 39.4 ± 13.6$^{a}$ | .059 ± .002$^{ac}$ |
| | 8 | 2.46 ± 3.47$^{ab}$ | 23.24 ± 9.17$^{de}$ | 43.2 ± 9.7$^{ab}$ | .060 ± .001$^{ac}$ |
| | 10 | 10.39 ± .74$^{ij}$ | 24.70 ± 5.70$^{dg}$ | 36.2 ± 2.9$^{a}$ | .057 ± .002$^{ac}$ |
| | 12 | 11.66 ± 1.08$^{jk}$ | 26.51 ± 5.58$^{dg}$ | 46.4 ± 4.9$^{ab}$ | .059 ± .003$^{ac}$ |
| | 14 | 12.09 ± 1.78$^{jk}$ | 23.88 ± 6.84$^{df}$ | 72.2 ± 12.3$^{ab}$ | .063 ± .003$^{ce}$ |
| | 16 | 13.08 ± 1.93$^{kl}$ | 26.74 ± 3.80$^{dg}$ | 71.5 ± 19.4$^{ab}$ | .064 ± .004$^{ce}$ |
| | 18 | 14.71 ± 1.43$^{lm}$ | 27.83 ± 1.59$^{dg}$ | 90.5 ± 51.9$^{ab}$ | .061 ± .010$^{bc}$ |
| | 20 | 15.98 ± 1.20$^{m}$ | 32.06 ± 3.34$^{g}$ | 137.5 ± 112.5$^{abc}$ | .062 ± .004$^{ce}$ |
| | 22 | 13.71 ± 1.56$^{km}$ | 33.25 ± .26$^{g}$ | 330.6 ±388.4$^{e}$ | .059 ± .002$^{ac}$ |
| | 24 | 15.00 ± .51$^{jm}$ | 30.78 ± 2.88$^{feg}$ | 52.0 ± .09$^{ab}$ | .054 ± .012$^{a}$ |

a,b,c,d,e,f,g,h,i,j,k,l,m Means ± S.D. followed by the same superscript within the same column are not significantly different ($p > .05$)

TABLE 9. Changes of physicochemical properties in the 3.0x concentrated whole milk (2500/500psi homogenization pressure) from two different membrane processings (multiple-membrane system and RO single membrane) during 24 weeks' storage.

| Membrane processing | Time (week) | Creaming (%) | Sedimentation (%) | Viscosity (cp) | NPN (%) |
|---|---|---|---|---|---|
| RO membrane | 2 | .00 ± .00$^{a}$ | 5.74 ± 6.68$^{a}$ | 160.5 ± 13.7$^{c}$ | .067 ± .003$^{ghij}$ |
| | 4 | .00 ± .00$^{a}$ | 16.21 ± .77$^{e}$ | 203.5 ± 18.0$^{def}$ | NM |
| | 6 | .00 ± .00$^{a}$ | 18.49 ± .90$^{e}$ | 220.0 ± 27.1$^{eg}$ | NM |
| | 8 | .00 ± .00$^{a}$ | 15.47 ± .30$^{de}$ | 285.0 ± 27.2$^{ij}$ | .064± .001$^{dfg}$ |
| | 10 | 3.67 ± .44$^{b}$ | 11.23 ± .58$^{bc}$ | 197.5 ± 9.2$^{def}$ | .062 ± .000$^{de}$ |
| | 12 | 4.20 ± .46$^{b}$ | 12.53 ± 1.75$^{cd}$ | 244.0 ±14.0$^{gh}$ | .061 ± .002$^{cd}$ |
| | 14 | 4.85 ± .54$^{b}$ | 11.45 ± .37$^{bc}$ | 265.5 ± 23.8$^{hi}$ | .068 ± .002$^{ij}$ |
| | 16 | 5.90 ± 1.23$^{b}$ | 11.96 ± 3.71$^{bc}$ | 310.0 ± 18.8$^{j}$ | .074 ± .005$^{k}$ |
| | 18 | 5.10 ± .37$^{b}$ | 8.65 ± 2.87$^{ab}$ | 429.5 ± 46.9$^{k}$ | .065 ± .002$^{efi}$ |
| | 20 | 5.39 ± .95$^{b}$ | 10.37 ± .82$^{bc}$ | 494.0 ± 46.1$^{l}$ | .065 ± .003$^{fj}$ |
| | 22 | 5.19 ± 1.26$^{b}$ | 10.05 ± 2.12$^{bc}$ | 508.5 ± 56.1$^{l}$ | .067 ± .003$^{hij}$ |
| | 24 | 5.38 ± .65$^{b}$ | 11.67 ± .69$^{bc}$ | gel | .061 ± .001$^{cd}$ |
| Multiple membrane | 2 | 4.41 ± 5.09$^{b}$ | 36.06 ± 1.19$^{hjk}$ | 54.5 ± .6$^{a}$ | .065 ± .002$^{efi}$ |
| | 4 | 5.35 ± 6.18$^{b}$ | 36.22 ± 2.76$^{hjk}$ | 54.0 ±13.2$^{a}$ | .064 ± .003$^{efh}$ |
| | 6 | 5.27 ± 6.17$^{b}$ | 29.22 ± 5.02$^{f}$ | 71.5 ± 3.4$^{a}$ | .058 ± .001$^{c}$ |
| | 8 | 12.66 ± .47$^{c}$ | 34.66 ± .12$^{gij}$ | 60.3 ± 4.0$^{a}$ | .065 ± .000$^{efi}$ |
| | 10 | 12.39 ± 2.03$^{c}$ | 32.69 ± 1.00$^{g}$ | 80.0 ±14.2$^{ab}$ | .053 ± .001$^{b}$ |
| | 12 | 12.74 ± .80$^{c}$ | 33.35 ± .62$^{gh}$ | 79.5 ± 9.8$^{ab}$ | .041 ± .002$^{a}$ |
| | 14 | 13.89 ± 1.95$^{cd}$ | 33.72 ± 1.05$^{gh}$ | 74.5 ±10.5$^{a}$ | .050 ± .003$^{b}$ |
| | 16 | 15.37 ± .70$^{cd}$ | 33.76 ± .16$^{gh}$ | 84.5 ± 26.7$^{ab}$ | NM |
| | 18 | 14.20 ± 1.05$^{cd}$ | 32.42 ± 1.23$^{fg}$ | 111.5 ±32.8$^{b}$ | .063 ± .000$^{df}$ |
| | 20 | 14.90 ± 1.12$^{cd}$ | 34.92 ± 2.11$^{gij}$ | 174.0 ±13.2$^{cd}$ | .063 ± .002$^{df}$ |
| | 22 | 16.23 ± .51$^{d}$ | 37.73 ± .67$^{ik}$ | 187.0 ±30.6$^{ce}$ | .068 ± .003$^{j}$ |
| | 24 | 16.18 ± 1.08$^{d}$ | 34.72 ± 3.67$^{gij}$ | 223.5 ±13.1$^{gh}$ | .068 ± .003$^{ij}$ |

$^{a,b,c,d,e,f,g,h,i,j,k,l}$ Means ± S.D. followed by the same superscript within the same column are not significantly different ($P > .05$)

NM indicates no measurement.

TABLE 10. ANOVA of a three-factor factorial design in sedimentation of the concentrated whole milk over 24 weeks' storage.

| Source | DF | MS | F ratio | Prob >F |
|---|---|---|---|---|
| Concentration level (C) | 2 | 11458.703 | 423.511 | .0000 |
| Homogenization pressure (HP) | 3 | 6.652 | .246 | .8643 |
| C*HP | 6 | 365.981 | 13.527 | .0000 |
| Storage time (ST) | 11 | 232.660 | 8.599 | .0000 |
| C*ST | 22 | 154.159 | 5.698 | .0000 |
| HP*ST | 33 | 34.940 | 1.291 | .1339 |
| C*HP*ST | 66 | 53.549 | 1.979 | .0000 |
| Error | 422 | 27.056 | | |

happened on sedimentation under homogenization pressures in the 3.0x concentrated whole milk (Table 6). Sedimentation causes a serious problem in all concentrated milk. In the 2.5x concentrated milk the average sediment was 17.78%, which represented almost one fifth the total volume. Sedimentation occurred in all milk concentration levels during 6 months' storage as shown in Figures 9, 10, and 11. After the 2nd or 4th wk, the sedimentation was from 17% to 38% in all multiple-membrane concentrated whole milks. This phenomenon indicated storage time effect on the rapid sedimentation could be replaced by other detrimental factors.

Concentrated whole milk produced by the RO membrane under low homogenization pressure had the lowest sedimentation at all concentration levels. There was significantly different sedimentation at the three concentration levels between RO milk and MM milk during 6 months (Tables 7, 8, and 9). The reason for the difference in sedimentation between them probably was the

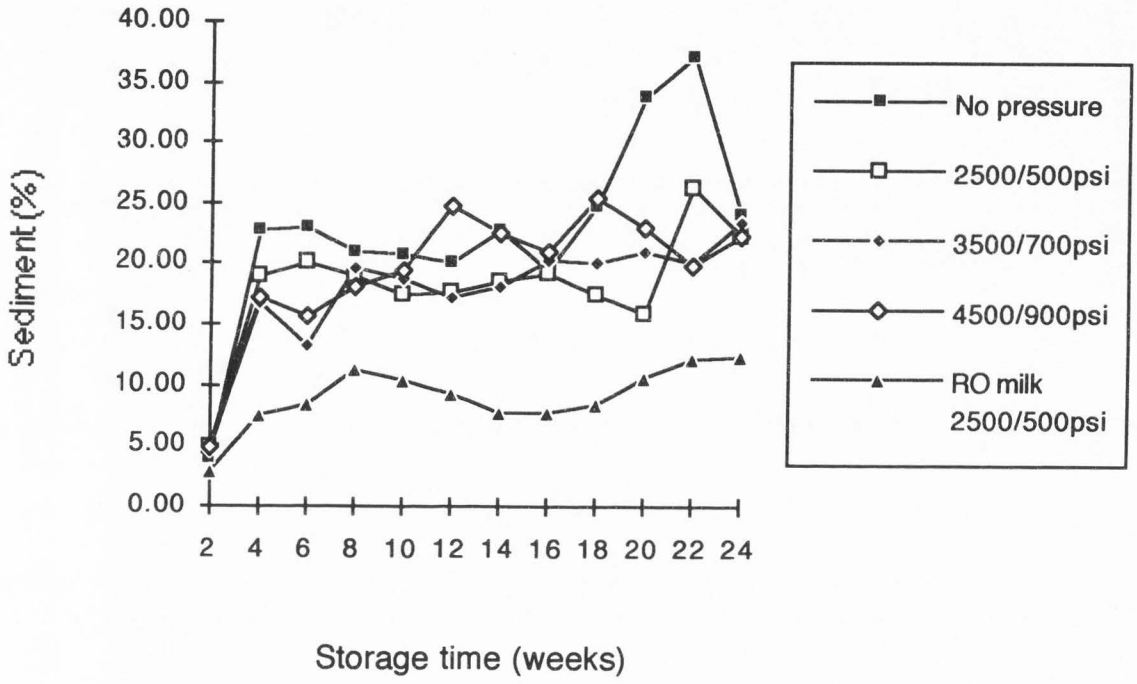


Figure 9. Changes of sedimentation in 2.5x concentrated whole milk with four different homogenization pressures and RO milk as the control during 24 weeks' storage.

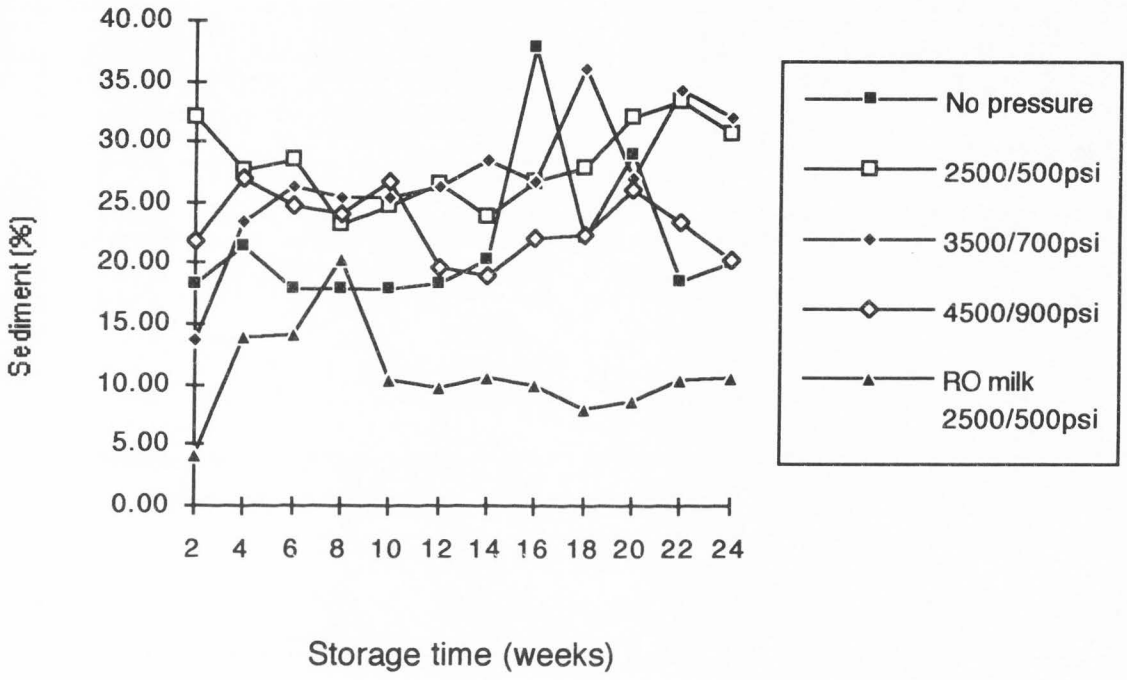


Figure 10. Changes of sedimentation in 2.75x concentrated whole milk with four different homogenization pressures and RO milk as the control during 24 weeks' storage.

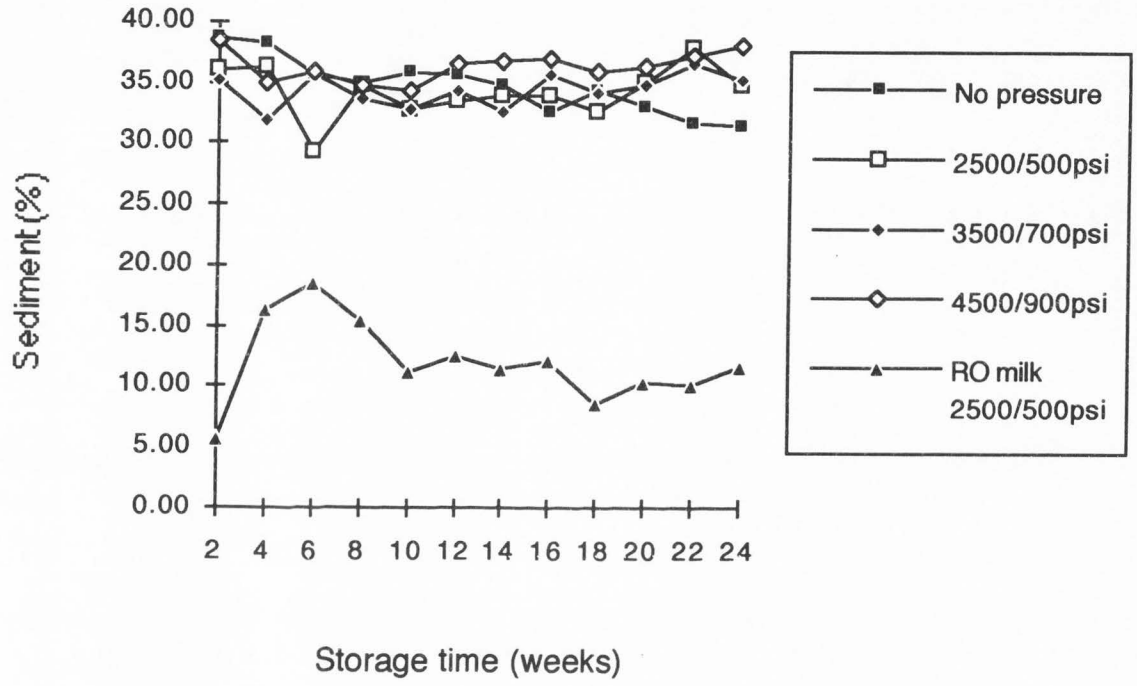


Figure 11. Changes of sedimentation in 3.0x concentrated whole milk with four different homogenization pressures and RO milk as the control during 24 weeks' storage.

disturbance in the integrity of milk components system during membrane processing. That means recombination of UF retentate and RO retentate could have destablized the relation between casein micelles and minerals, particularly calcium. The equilibrium of components in original milk is probably not reestablished in the concentrated whole milk produced from the multiple-membrane system. Dalgleish (22) suggested that in stored milk sedimentation is a purely physical effect under undisturbed conditions and easy to eliminate by agitation, but may be accelerated in the concentrated milk. One possible assumption is the deposition of calcium phosphate onto the destablized surface of casein micelles, which causes an increase of the micelle weight followed by sedimentation. Moreover, the aggregation of the modified casein micelles will contribute to the rapid sedimentation (3, 22, 76, 77). The actual mechanism that causes the rapid and heavy sedimentation in the concentrated whole milk from the multiple-membrane system remains to be solved.

**Viscosity**

Viscosity is a fluid property that measures the resistance of fluids to shear (11). In concentrated whole milk, viscosity is possibly influenced by the degree of sedimentation. A sediment can contain considerable solids, and viscosity of milk depends on how many solids remain in the liquid phase. Age gelation is a common storage defect in UHT milk products and may be treated as an infinite viscosity in a gelled product. There were not enough data on concentration levels effect and could not be tested to its fullest extent. Therefore, in order to avoid the degree of freedom decreasing due to the difficulty of the viscosity measurement in the gelled products, the viscosity value was given 2000 cps instead of the blank. The ANOVA test of viscosity of the concentrated whole milk is presented in Table 11.

TABLE 11. ANOVA of a three-factor factorial design in viscosity of the concentrated whole milk over 24 weeks' storage.

| Source | DF | MS | F ratio | Prob >F |
|---|---|---|---|---|
| Concentration level (C) | 2 | 604616 | 7.695 | .0005 |
| Homogenization pressure (HP) | 3 | 5077718 | 64.627 | .0000 |
| C*HP | 6 | 36341 | .463 | .8360 |
| Storage time (ST) | 11 | 3540363 | 45.060 | .0000 |
| C*ST | 22 | 190036 | 2.419 | .0004 |
| HP*ST | 33 | 1037509 | 13.205 | .0000 |
| C*HP*ST | 66 | 73656 | .938 | .6166 |
| Error | 432 | 78569 | | |

Homogenization pressure did not affect viscosity of the concentrated whole milk at the three concentration levels (Table 6). The high standard deviation certain amount of solids in the sediment caused the MM milk to be more aqueous than the control RO milk. Consequently, viscosity is lower in the MM milk than in the RO milk. Gelation only occurred in the MM milk with no homogenization pressure and in the RO milk with low homogenization pressure after 14 wk. Because the high sedimentation could cause a small portion of casein micelles and salts to remain in the liquid phase, it is possible the MM milk cannot form a gel structure at the end of 24 weeks except for the MM milk with no homogenization pressure. One possible explanation for this exception is that no homogenization pressure caused more cream plug that accumulated throughout the storage time leading to gel network formation. This phenomenon is similar to fat globules in creaming, forming a continuous network by cold agglutination (78).resulted from the large range of viscosity during 24 weeks' measurement. Viscosity gradually increased in both RO milk

and MM milk during 24 weeks' storage (Figures 12, 13, and 14). In UHT milk studies, this increase in viscosity with time was due to denaturation and unfolding of proteins (4, 28). Increasing viscosity is an indication of approaching gelation (36, 48). It appears that all multiple-membrane concentrated whole milk maintained lower viscosity over 24 weeks' storage compared with RO concentrated whole milk. However, these experimental milks had greater sedimentation over the storage period so viscosity values may not indicate a uniform milk composition. We found that a certain amount of solids in the sediment caused the MM milk to be more aqueous than the control RO milk. Consequently, viscosity is lower in the MM milk than in the RO milk. Gelation only occurred in the MM milk with no homogenization pressure and in the RO milk with low homogenization pressure after 14 wk. Because the high sedimentation could cause a small portion of casein micelles and salts to remain in the liquid phase, it is possible the MM milk cannot form a gel structure at the end of 24 weeks except for the MM milk with no homogenization pressure. One possible explanation for this exception is that no homogenization pressure caused more cream plug that accumulated throughout the storage time leading to gel network formation. This phenomenon is similar to fat globules in creaming, forming a continuous network by cold agglutination (78).

**Nonprotein Nitrogen**

The ANOVA test of NPN content in the concentrated whole milk over 24 weeks' storage is presented in Table 12. There were two missing measurements during 24 week's survey. Linear regression was used to estimate the missing data so that the degree of freedom would not decrease and the ANOVA could be tested completely. In the 2.5x and 2.75x concentrated whole milks, the medium homogenization pressure caused the milk products to

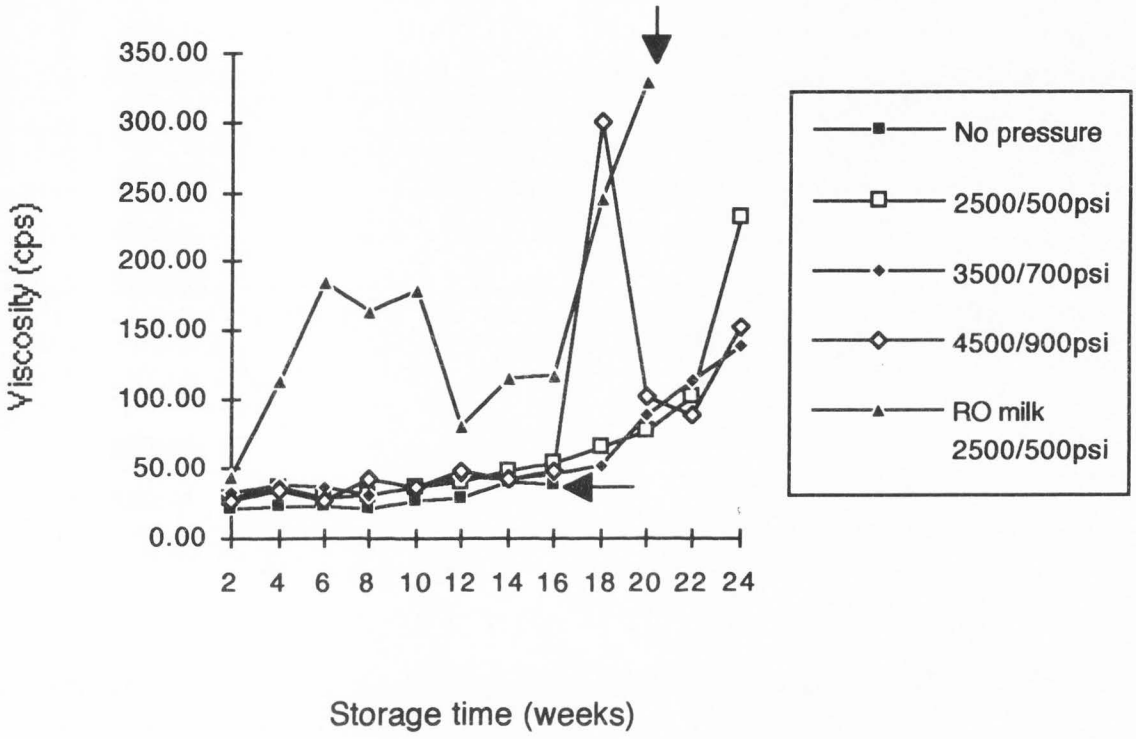


Figure 12. Changes of viscosity in 2.5x concentrated whole milk with four different homogenization pressures and RO milk as the control during 24 weeks' storage. Arrow points out the time in UHT concentrated whole milk which was beginning to gelation.

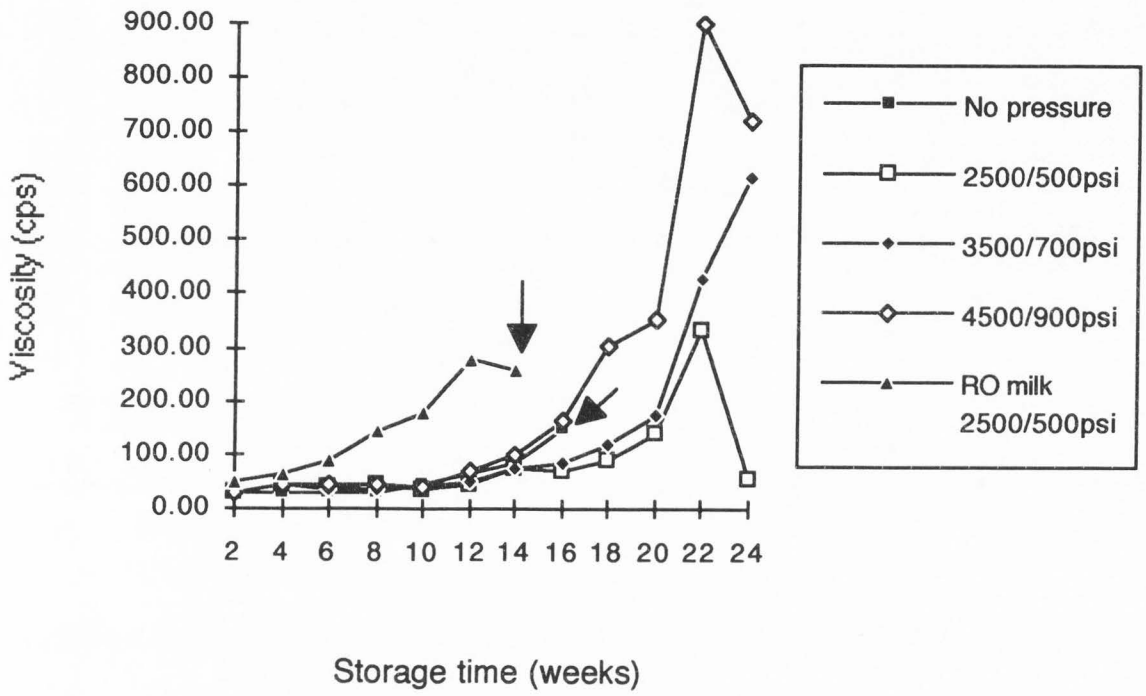


Figure 13. Changes of viscosity in 2.75x concentrated whole milk with four different homogenization pressures and RO milk as the control during 24 weeks' storage. Arrow points out the time in UHT concentrated whole milk which was beginning to gelation.

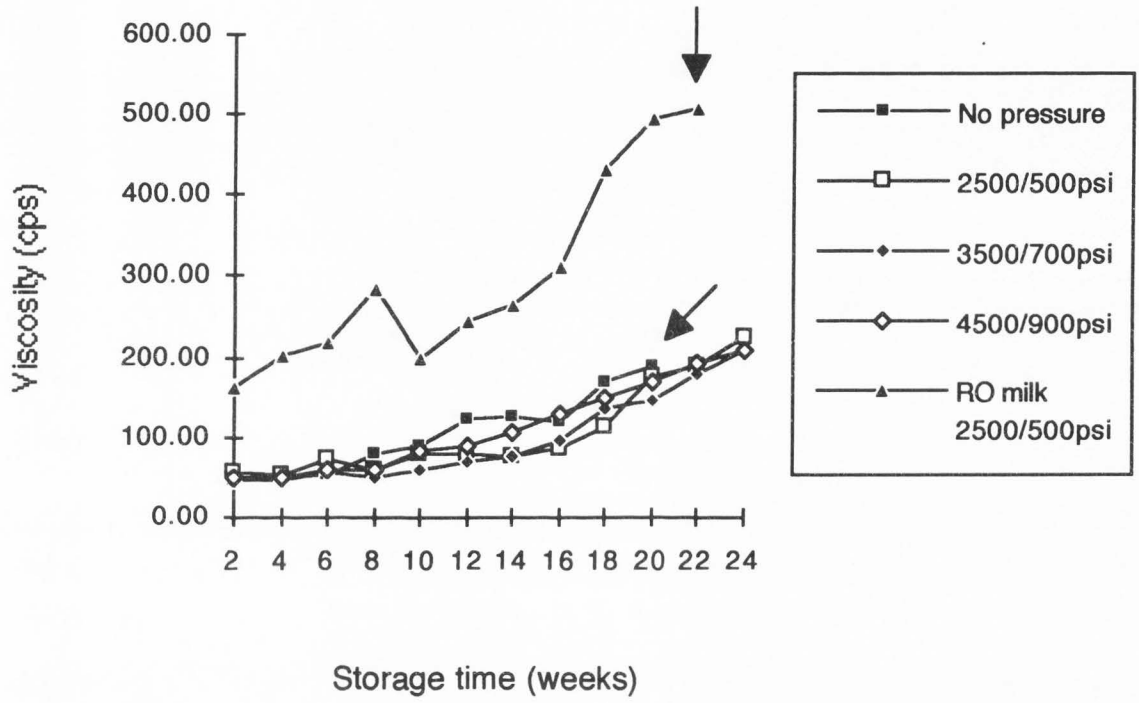


Figure 14. Changes of viscosity in 3.0x concentrated whole milk with four different homogenization pressures and RO milk as the control during 24 weeks' storage. Arrow points out the time in UHT concentrated whole milk which was beginning to gelation.

TABLE 12. ANOVA of a three-factor factorial design in NPN content of the concentrated whole milk over 24 weeks' storage.

| Source | DF | MS | F ratio | Prob >F |
|---|---|---|---|---|
| Concentration level (C) | 2 | .000205 | 29.513 | .0000 |
| Homogenization pressure (HP) | 3 | .000053 | 11.966 | .0000 |
| C*HP | 6 | .000012 | 4.534 | .0002 |
| Storage time (ST) | 11 | .000189 | 9.113 | .0000 |
| C*ST | 22 | .000234 | 8.731 | .0000 |
| HP*ST | 33 | .000026 | .931 | .5808 |
| C*HP*ST | 66 | .000031 | 1.196 | .1546 |
| Eorror | 412 | .000027 | | |

lose from 8 to 9% NPN. The low and high homogenization pressures made no difference in NPN content compared with the nonhomogenized control. No significant change of NPN content occurred in the 3.0x concentrated whole milk under all homogenization pressures (Table 6). Loss of NPN under homogenization pressures is not clear. Walstra and Jenness (77) suggest that heat treatments such as pasteurization and possibly UHT do not cause detectable increases in NPN content. During storage, Aoki and Imamura (4) found that NPN content of sterilized skim milk (135°C for 45 sec) increased from 36 mg/100 ml to 49 mg/100 ml after 5 months' storage. Harwalkar et al. (36) also reported an increase in NPN content of sterilized concentrated skim milk at 28° C during 18 weeks' storage.

The changes of NPN content in the concentrated whole milk during 24 weeks are shown in Tables 13, 14, and 15. The initial NPN content in all concentrated whole milks served as a base line to compare with other NPN content

TABLE 13. Changes of NPN content in 2.5x concentrated whole milk with four different homogenization pressures during 24 weeks' survey.

| Storage time | Homogenization pressure | | | |
|---|---|---|---|---|
| (week) | no pressure | 2500/500 psi | 3500/700psi | 4500/900psi |
| 2 | .055 ± .003[a] | .056 ± .004[a] | .053 ± .003[a] | .055 ± .003[ab] |
| 4 | .056 ± .003[a] | .052 ± .001[a] | .046 ± .001[a] | .049 ± .008[a] |
| 6 | .059 ± .001[a] | .058 ± .001[a] | .056 ± .002[a] | .057 ± .001[ab] |
| 8 | .058 ± .001[a] | .056 ± .001[a] | .055 ± .005[a] | .055 ± .002[ab] |
| 10 | .057 ± .001[a] | .058 ± .001[a] | .055 ± .000[a] | .056 ± .000[ab] |
| 12 | .061 ± .006[a] | .059 ± .006[a] | .059 ± .004[a] | .057 ± .003[ab] |
| 14 | .060 ± .006[a] | .059 ± .004[a] | .057 ± .002[a] | .055 ± .003[ab] |
| 16 | .065 ± .009[a] | .062 ± .005[a] | .059 ± .003[a] | .059 ± .002[ab] |
| 18 | .064 ± .005[a] | .064 ± .006[a] | .060 ± .003[a] | .060 ± .002[ab] |
| 20 | .069 ± .008[a] | .063 ± .005a | .060 ± .003[a] | .065 ± .007[b] |
| 22 | .063 ± .005[a] | .062 ± .004[a] | .059 ± .000[a] | .065 ± .006[b] |
| 24 | .063 ± .018[a] | .056 ± .014[a] | .049 ± .015[a] | .055 ± .009[ab] |

[a,b] Mean ± S.D. followed by the same superscript within the same column are not significantly different ($P > .05$).

TABLE 14. Changes of NPN content in 2.75x concentrated whole milk with four different homogenization pressures during 24 weeks' survey.

| Storage time | Homogenization pressure | | | |
|---|---|---|---|---|
| (week) | no pressure | 2500/500 psi | 3500/700psi | 4500/900psi |
| 2 | .060 ± .005[a] | .061 ± .006[b] | .057 ± .007[a] | .058 ± .003[ab] |
| 4 | .066 ± .003[a] | .065 ± .005[b] | .058 ± .001[a] | .057 ± .006[ab] |
| 6 | .056 ± .003[a] | .059 ± .002[ab] | .056 ± .002[a] | .059 ± .006[ab] |
| 8 | .063 ± .002[a] | .060 ± .001[ab] | .055 ± .001[a] | .062 ± .002[ab] |
| 10 | .062 ± .003[a] | .057 ± .002[ab] | .058 ± .003[a] | .071 ± .001[b] |
| 12 | .065 ± .005[a] | .059 ± .003[ab] | .057 ± .002[a] | .059 ± .002[ab] |
| 14 | .064 ± .006[a] | .063 ± .003[b] | .061 ± .002[a] | .062 ± .003[ab] |
| 16 | .064 ± .007[a] | .064 ± .004[b] | .060 ± .000[a] | .063 ± .004[ab] |
| 18 | .066 ± .005[a] | .061 ± .010[b] | .060 ± .002[a] | .060 ± .005[ab] |
| 20 | .067 ± .009[a] | .062 ± .004[b] | .060 ± .001[a] | .063 ± .006[ab] |
| 22 | .065 ± .006[a] | .059 ± .002[ab] | .058 ± .002[a] | .061 ± .006[ab] |
| 24 | .060 ± .009[a] | .054 ± .012[a] | .055 ± .008[a] | .049 ± .017[a] |

[a,b] Mean ± S.D. followed by the same superscript within the same column are not significantly different ($P > .05$)

TABLE 15 . Changes of NPN content in 3.0x concentrated whole milk with four different homogenization pressures during 24 weeks' survey.

| Storage time | Homogenization pressure | | | |
|---|---|---|---|---|
| (week) | no pressure | 2500/500 psi | 3500/700psi | 4500/900psi |
| 2 | .064 ± .001$^{ab}$ | .065 ± .002$^{a}$ | .064 ±.002$^{be}$ | .064 ± .003$^{abc}$ |
| 4 | .063 ± .001$^{ab}$ | .064 ± .003$^{a}$ | .064 ±.000$^{be}$ | .063 ± .002$^{abc}$ |
| 6 | .063 ± .001$^{ab}$ | .058 ± .001$^{b}$ | .062 ±.002$^{be}$ | .061 ± .004$^{abc}$ |
| 8 | .063 ± .001$^{ab}$ | .065 ± .000$^{a}$ | .067 ±.002$^{be}$ | .063 ± .003$^{abc}$ |
| 10 | .061 ± .008$^{ab}$ | .053 ± .001$^{bc}$ | .060± .013$^{abc}$ | .059 ± .010$^{ab}$ |
| 12 | .057 ± .002$^{a}$ | .041 ± .002$^{d}$ | .048 ±.005$^{a}$ | .056 ± .001$^{a}$ |
| 14 | .066 ± .001$^{b}$ | .050 ± .003$^{c}$ | .055 ±.007$^{ab}$ | .061 ± .001$^{abc}$ |
| 16 | .067 ± .003$^{b}$ | NM | .061± .002$^{bd}$ | .065 ± .001$^{abc}$ |
| 18 | NM | .063 ± .000$^{a}$ | .070± .001$^{cde}$ | .070 ± .002$^{be}$ |
| 20 | .057 ± .001$^{a}$ | .063 ± .002$^{a}$ | .066 ±.000$^{be}$ | .059 ± .009$^{ab}$ |
| 22 | .067 ± .004$^{b}$ | .068 ± .003$^{a}$ | .070± .006$^{cde}$ | .071 ± .001$^{cde}$ |
| 24 | .068 ± .007$^{b}$ | .068 ± .003$^{a}$ | .071± .004$^{cde}$ | .070 ± .004$^{bd}$ |

$^{a,b,c,d,e}$ Mean ± S.D. followed by the same superscript within the same column are not significantly different ($p > .05$)
NM indicates no measurement.

measurements during 24 weeks' storage. In the 2.5x and 2.75x concentrated whole milks, NPN content did not change significantly under all homogenization pressures (low, medium, high, and no homogenization pressures) during 24 wk. At the end of 6 mo, however, NPN content decreased significantly under low homogenization pressure in the 2.75x concentrated whole milk. In the 3.0x concentrated whole milk, no significant changes occurred under the high and no homogenization pressures. Nonprotein nitrogen content decreased from the 6th week to the 14th week under the low homogenization pressure. Under the medium homogenization pressure, NPN content only decreased at the 12th week.

Nonprotein nitrogen is mainly comprised of urea, creatine, small peptides, amino acids, and other minor nitrogen compounds (37). If any proteolysis

activity occurred in the concentrated whole milk during storage, NPN content will increase due to protein degradation. Neither microbial contamination nor proteinase activity occurred in the UHT milk products that resulted in NPN content remaining constant during 24 weeks' storage.

Most treatments did not cause changes in NPN content in the 2.5x and 2.75x concentrated whole milk (Tables 13, 14, and 15). However, we observed that NPN content decreased rather than increased in some treatments of the 3.0x concentrated whole milk. The possible experimental errors of nitrogen determination by Kjeldahl procedure are taken into consideration and gelation or sedimentation also could have caused the difficulty of homogenized sampling.

The concentrated whole milk can be successfully produced by the multiple-membrane system. It is feasible to commercialize this membrane concentration system. The concentrated milks given the UHT treatment could face some physicochemical changes such as sedimentation during storage. Further research in UHT processing conditions or proper pretreatment of the product before UHT is required for the long shelf life and desirable qualities of the final products.

# CONCLUSIONS

1. The 2.5x, 2.75x, and 3.0x concentrated whole milks produced by the multiple-membrane concentration system could result in target concentrations of all major nutrients except nonprotein nitrogen.

2. Twenty percent to 32% of nonprotein nitrogen was lost during RO concentration processing in the production of the multiple-membrane concentrated whole milk and RO milk.

3. All multiple-membrane concentrated whole milks at all homogenization pressures had greater sedimentation than the RO concentrated milk control.

4. Homogenization pressure could not efficiently prevent sedimentation in all multiple-membrane concentrated whole milk.

5. All multiple-membrane concentrated whole milks with all homogenization pressure had less cream plug formation than without homogenization pressure over 24 weeks' storage.

6. A higher homogenization pressure can delay cream plug formation longer.

7. All multiple membrane concentrated whole milks maintained lower viscosity over 24 weeks' storage compared with RO concentrated whole milk. However, these experimental milks had greater sedimentation over the storage period so the viscosity values may not indicate a uniform milk composition.

8. All concentrated whole milks gradually increased viscosity during 24 weeks' storage.

9. Viscosity was not affected by homogenization pressure in all concentrated whole milks.

10. Nonprotein nitrogen content did not significantly change in the 2.5x, 2.75x, and 3.0x concentrated whole milks during the 24 weeks' storage period.

## REFERENCES


1 Adams, D. M., J. T. Barach, and M. L. Speck. 1976. Effect of psychrotrophic bacteria from raw milk on milk proteins and stability of milk protein to ultrahigh temperature treatment. J. Dairy Sci. 59: 823.

2 Andrew, A T., B. E. Brooker, and D.G. Hobbs. 1977. Properties of aseptically packed ultra-high-temperature milk. Electron microscopic examination of changes occurring during storage. J. Dairy Res. 44:283.

3 Andrew, A T., and G. C. Cheeseman. 1972. Properties of aseptically packed ultra-high-temperature milk.II. Molecular weight changes of casein components during storage. J. Dairy Res. 39:395.

4 Aoki, T., and T. Imamura. 1974. Changes of casein complex during storage of sterilized skim milk. Agr. Biol. Chem. 38:1929.

5 Ashton, T. R. 1965. Practical experience: the processing and aseptic packaging of sterile milk in the United Kingdom. J. Soc. Dairy Technol. 18:65.

6 Association of Official Analytical Chemists. 1990. Official methods of analysis. Vol. I. 15th ed. AOAC, Arlington, VA.

7 Badings, H. T. 1977. A method of preventing the occurrence of cooked flavor in milk and milk products. Dairy Sci. Abstr. 40:6121.

8 Barbano, D. M., V. Sciancalepore, and M. A. Rudan. 1988. Characterization of milk proteins in ultrafiltration permeate. J. Dairy Sci. 71: 2655.

9 Barbano, D. M., M. A. Rudan, and R. R. Rasmussen. 1989. Influence of milk composition and somatic cell and psychrotropic bacteria counts on ultrafiltration flux. J. Dairy Sci. 72:1118.

10 Bastian, E. D., S. K. Collinge, and C. A. Ernstrom. 1991. Ultrafiltration: Partitioning of milk constituents into permeate and retentate. J. Dairy Sci. 74:2423.

11 Batty, J. C., and S. L. Folkman. 1983. How fluids flow. Page 143 *in* Food engineering fundamentals. J. C. Batty and S. L. Folkman, ed. John Wiley & Sons, Inc. New York. NY.

12 Boekel, M. A. J. S. V., and P. Walstra. 1989. Physical changes in the fat globules in unhomogenized and homogenized milk. Annu. Bull. Int. Dairy Fed. 238:13.

13 Brunner, J. R. 1974. Physical equilibria in milk: the lipid phase. Page 474 *in* Fundamentals of dairy chemistry. 2nd ed. B. H. Webb, A. H. Johnson, and J. A. Alford, ed. The AVI Publishing Company, Inc. Westport, CT.

14 Burton, H. 1968. Reviews of the progress of Dairy Science. Section G. Deposits from whole milk in heat treatment plant-- a review and discussion. J. Dairy Res. 35:317.

15 Burton, H. 1977. An introduction to ultra-high-temperature processing and plant. J. Soc. Dairy Technol. 30:135.

16 Burton, H. 1988. Types of UHT processing plant. Page 77 *in* Ultra-high-temperature processing of milk and milk products. H. Burton, ed. Elsevier Applied Science, New York, NY.

17 Carroll, R. J., M. P. Thompson, and P. Melnychyn. Gelation of concentrated skimmilk: electron microscopic study. J. Dairy Sci. 54:1245.

18 Cheng, W.S., and C. S. Gelda. 1975. Effects of double homogenization on ultra pasteurized 10% cream. J. Dairy Sc. 58:800.

19 Cotton, S. G. 1980. The utilization of permeates from the ultrafiltration of whey and skim milk. J. Soc. Dairy Technol. 33:89.

20 Covacevich, H. R., and F. V. Kosikowski. 1977. Cream cheese by ultrafiltration. J. Food Sci. 42:1362.

21 Cox, G. C., and I. A. Langdon. 1985. Economic evaluation of reverse osmosis for reduction in milk transport costs. Aust. J. Dairy Technol. 40:113.

22 Dalgleish, D. G. 1991. Sedimentation of casein micelles during the storage of ultra-high temperature milk products--a calculation. J. Dairy Sci. 75: 371.

23 Dix, B. D. 1985. Dairy products prepared from reverse osmosis concentrate--market milk products, butter, skim milk powder, and yoghurt. Aust. J. Dairy Technol. 40:91.

24 Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

25 El-Gazzar, F. E., and E. E. Marth. 1991. Ultrafiltration and reverse osmosis in dairy technology: a review. J. Food Prot. 54:801.

26 Ernstrom, C. A., B. J. Sutherland, and G. W. Jameson. 1980. Cheese base for processing. A high yield product from whole milk by ultrafiltration. J. Dairy Sci. 63:228.

27 Ferretti, A., L. F. Edmondson, and F. W. Douglas, Jr. 1974. Control of cooked flavor in high-temperature short-time milk concentrates with a sulfhydryl-blocking agent. J. Agr. Food Chem. 22:1130.

28 Ferry, J. D. 1948. Protein gels. Adv. Protein Chem. 4:1.

29 Fischbach-Greene, L., and N. N. Potter. 1986. Effects of ultrafiltration on retention of minerals and other components of milk. J. Food Sci. 51:345.

30 Ford, J. E. 1957. Factors influencing the destruction by heat of vitamin B12 in milk. J. Dairy Res. 24:360.

31 Ford, J. E. 1967. The influence of the dissolved oxygen in milk on the stability of some vitamins towards heating and during subsequent exposure to sunlight. J. Dairy Res.34:239.

32 Glover, F. A. 1971. Concentration of milk by ultrafiltration and reverse osmosis. J. Dairy Res. 38:373.

33 Glover, F.A. 1986. Modifications to the composition of milk. Page 235 *in* Modern dairy technology. Vol. 1. R. K. Robinson. ed. Elsevier Applied Science Publishing Co., Inc. New York, NY.

34 Gooding, C. H. 1985. Reverse osmosis and ultrafiltration solve separation problems. Chem. Eng. 92:56.

35 Green, M. L., K. J. Scott, M. Anderson, M. C. A. Griffin, and F. A. Glover. 1984. Chemical characterization of milk concentration by ultrafiltration. J. Dairy Res. 51:267.

36 Harwalkar, V. R., and H. J. Vreeman. 1978. Effect of added phosphates and storage on changes in ultra-high temperature short-time sterilized concentrated skim-milk. 1. Viscosity, gelation, alcohol stability, chemical and electrophoretic analysis of proteins. Neth. Milk Dairy J. 32:94.

37 Jenness, R. 1988. Composition of milk. Page 1 *in* Fundamentals of dairy chemistry. 3rd ed. N. P. Wong, ed. Van Nostrand Reinhold Company, New York, NY.

38 Jenness, R., and S. Patton. 1959. The physical chemistry of milk fat globules. Page 265 *in* Principles of dairy chemistry. R. Jenness and S. Patton, ed. John Wiley & Sons, Inc. New York, NY.

39 Jenness, R., and S. Patton. 1959. Flavors and off flavors in milk and its products. Page 265 *in* Principles of dairy chemistry. R. Jenness and S. Patton, ed. John Wiley & Sons, Inc. New York, NY.

40 Jones, V. A., and W. J. Harper. 1976. General processes for fluid milks. Page 141 *in* Dairy technology and engineering. W. J. Harper and C. W. Hall, ed. The AVI Publishing Company, Inc., Westport, CT.

41 King, N. 1955. The milk fat globule membrane. Commonwealth Agricultural Bureau, Farnham Royal, Bucks, England.

42 Kocak, H. R. 1985. Dairy products manufactured from whole milk concentrated by reverse osmosis part I--UHT products. Aust. J. Dairy Technol. 40:96.

43 Koops, J. 1967. Preparation and properties of sterilized coffee cream II. continuous-flow sterilization and aseptic packaging. Neth. Milk & Dairy J. 21:50.

44 Kosikowski, F. 1986. New cheese-making procedures utilizing ultrafiltration. Food Technol. 40:71.

45 Kulozik, U., and H. G. Kessler. 1988. Permeation rate during reverse osmosis of milk influenced by osmotic pressure and deposit formation. J. Food Sci. 53: 1377.

46 Law, B.A., A. T. Andrew, and M. E. Sharpe. 1977. Gelation of ultra-high-temperature-sterilized milk by proteases from a strain of Pseudomonas fluorescence isolated from raw milk. J. Dairy Res. 44:145.

47 LeMaire, W. H. 1985. Field report: ultrafiltration cuts milk costs for dairy farm, cheese plants. Food Eng. 57:178.

48 Lewis, M. J. 1986. Advances in the heat treatment of milk. Page 1 *in* Modern dairy technology. R.K. Robinson., ed. Elsevier Applied Science, New York, NY.

49 Marier, J. R., and M. Boulet. 1959. Direct analysis of lactose in milk and serum. J. Dairy Sci. 42:1390.

50 Marshall, S. C. 1985. The reverse osmosis process. Aust. J. Dairy Technol. 40:86.

51 Maubois, J-L., G. Mocquot, and L. Vassal. 1980. Preparation of cheese using ultrafiltration. U.S. Patent 4,205,080.

52 McBean, L., D. and E. W. Speckmann. 1988. Nutritive value of dairy foods. Page 343 *in* Fundamentals of dairy chemistry. 3rd ed. N. P. Wong, ed. Van Nostrand Reinhold Company, New York, NY.

53 Mehta, R. S. 1980. Milk processed at ultra-high-temperatures--a review. J. Food Prot. 43:212.

54 Mittal, S. B., J. A. Hourigan, J. G. Zadow, and M. H. Nguyen. 1988. Behaviour of UHT recombined and UHT recombined lactose hydrolyzed milk during storage at different temperatures. Aust. J. Dairy Technol. 43:64.

55 Muir, D. D. 1984. UHT-sterilized milk concentrate: a review of practical methods of production. J. Soc. Dairy Technol. 37:135.

56 Oamen, E. E. , A. P. Hansen, and K. R. Swartzel. 1989. Effect of ultra-high temperature steam injection processing and aseptic storage on labile water-soluble vitamins in milk. J. Dairy Sci. 72:614.

57 Paulson, D. J., R. L. Wilson, and D. D. Spatz. 1984. Crossflow membrane technology and its applications. Food Technol. 38:78.

58 Perkins, A. G., M.J. Henschel, and H. Burton. 1973. Comparison of milks processed by the direct and indirect methods of ultra-high-temperature sterilization. 6. Effects on sediment formation and clotting with enzymes. J. Dairy Res. 40:215.

59 Popper L., and D. Knorr. 1990. Applications of high-pressure homogenization for food preservation. Food Technol. 44: 84.

60 Porter, J. W. G., and S. Y. Thompson. 1972. The nutritive value of UHT milk. Annu. Bull. Int. Dairy Fed. 5:56.

61 Premaratne, R. J., and M. A. Cousin. 1991. Changes in the chemical composition during ultrafiltration of skim milk. J. Dairy Sci. 74:788.

62 Ramsey, J. A., and K. R. Swartzel. 1984. Effect of ultra high temperature processing and storage conditions on rates of sedimentation and fat separation of aseptically packaged milk. J. Food Sci. 49:257.

63 Renner, E. 1983. Milk & dairy products in human nutrition. Volkswirtschaftlicher Verlag, Munich, Germany.

64 Rowland, S. J. 1938. The determination of the nitrogen distribution in milk. J. Dairy Res. 9:42.

65 Samel, R., R. W. V. Weaver, and D.B. Gammack. 1971. Changes in storage in milk processed by ultra-high-temperature sterilization. J. Dairy Res. 38:323.

66 Samuelsson, E., G., and S. Holm. 1966. Technological properties for ultra-high-heat treatment of milk. 17th Int. Dairy Congress 2:57.

67 Samuelsson, E. G. 1971. Treating milk. U.S. Pat. 3623894. Official Gazette of United States Patent Office. Vol. 892. 5:1782.

68 Sawyer, W. H. 1968. Heat denaturation of bovine ß-lactoglobulins and relevance of disulfide aggregation. J. Dairy Sci. 51:323.

69 Schaafsma, G. 1989. Effects of heat treatment on the nutritional value of milk. Annu. Bull. Int. Dairy Fed. 238:68.

70 Schmidt K. A., and D. E. Smith. 1989. Effects of varying homogenization pressure on the physical properties of vanilla ice cream. J Dairy Sci. 72:378.

71 Shipe, W. F., R. Bassette, D. D. Deane, W L. Dunkley, E. G. Hammond, W. J. Harper, D.H. Kleyn, M. E. Morgan, J.H. Nelson, and R. A. Scanlan. 1978. Off flavors of milk: nomenclature, standards, and bibliography. J. Dairy Sci. 61:855.

72 Skudder, P. J. 1981. Effects of adding potassium iodate to milk before UHT treatment. I. Reduction in the amount of deposit on the heated surface. J. Dairy Res. 48:99.

73 Swaisgood, H. E., V. G. Janolino, and H. R. Horton. 1978. Immobilized sulfhydryl oxidase. AIChE Symposium Series 74 (172):25

74 Thomas, E. L., H. Burton, J. E. Ford, and A. G. Perkin. 1975. The effect of oxygen content on flavor and chemical changes during aseptic storage of whole milk after ultra-high-temperature processing. J. Dairy Res. 42:285.

75 Versteeg, C. 1985. Process and quality control aspects of reverse osmosis concentration of milk. Aust. J. Dairy Technol. 40:102.

76 Wahlgren, M., P. Dejmek, and T. Drakenberg. 1990. A 43Ca and 31P NMR study of the calcium and phosphate equilibria in heated milk solutions. J. Dairy Res. 57:355.

77 Walstra, P., and R. Jenness. 1984. Casein micelles. Page 229 *in* Dairy chemistry and physics. P. Walstra and R. Jenness, ed. John Wiley & Sons, New York, NY.

78 Walstra, P., and R. Jenness. 1984. Rheological properties. Page 290 *in* Dairy chemistry and physics. P. Walstra and R. Jenness, ed. John Wiley & Sons, New York, NY.

79 Walstra, P., and R. Jenness. 1984. Nutritive value. Page 358 *in* Dairy chemistry and physics. P. Walstra and R. Jenness, ed. John Wiley & Sons, New York, NY.

80 Weihranch J. L. 1988. Lipids of milk: deterioration. Page 215 *in* Fundamentals of dairy chemistry. 3rd ed. N. P. Wong, ed. Van Nostrand Reinhold Company, New York, NY.

81 Whitney, R. McL., and P. H. Tracy. 1949. Stale-flavor components in dried whole milk. I. The distribution of stale flavor between fractions of reconstituted stale whole milk powder. J. Dairy Sci. 32:383.

82 Wilson, H. K., E. O. Herreid, and R. M. Whitney. 1960. Ultra-centrifugation studies of milk heated to sterilization temperatures. J. Dairy Sci. 43:165.

83 Zadow, J. G. 1969. Studies on the ultra-heat treatment of milk. Part 1--Comparison of direct and indirect heating of whole milk. Aust. J. Dairy Technol. 24: 44.

84 Zall, R. R. 1984. Membrane processing of milk on the dairy farm. Food Technol. 38:88.

# APPENDIX

Table 16. Linear equation for estimating the missing data on NPN measurement.

| Missing data on NPN measurement | Linear equation | NPN Estimation |
|---|---|---|
| 3.0x conc. milk with no homogenization pressure at 18th week | y = .0001364x + .0615 | .0640% |
| 3.0x conc. milk with low homogenization pressure at 16th week | y = .0002227x + .05686 | .0604% |