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I am submitting herewith a thesis written by Aminta Martinez-Hermosilla entitled "Effect of Cottage Cheese Whey Pretreatment in the Production of Defatted Whey Protein Retentate using Two-phase Cross Flow Microfiltration and Ulltrafiltation." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Gregory J. Hulbert, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

EFFECT OF COTTAGE CHEESE WHEY PRETREATMENT IN THE PRODUCTION OF DEFATTED WHEY PROTEIN RETENTATE USING TWO-PHASE CROSS FLOW MICROFILTRATION AND ULTRAFILTRATION

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Aminta Martínez-Hermosilla
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DEDICATION

This thesis is dedicated to my husband and my children, whose love, support and constant attitude of concern will be forever treasured.

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ABSTRACT

This study investigated the effect of chemical and physical pretreatments in the permeate flux of cottage cheese whey during microfiltration and ultrafiltration, for the production of defatted whey retentates. Solutions of untreated and chemically treated whey were microfiltered through a 0.1μm pore size membrane at different flow rates, with and without the application of air to obtain the best combination that increased permeate flux by reducing membrane fouling. Significant differences ($p<0.05$) in permeate flux were found for RW at 10 L/min flow rate when either 10 or 20% air was injected during microfiltration. Application of 20% air significantly increased mean flux for EW. No significant difference ($p<0.05$) in flux was observed for CW when air was applied. Contrast statements also revealed that air significantly ($p<0.05$) increased flux across all types of whey during the microfiltration experiments. It was also found that protein permeation was significantly higher ($p<0.05$) when 20% air was used in the microfilter operating at 10 L/min liquid flow. Ultrafiltration of the microfiltration permeates revealed no significant difference ($p<0.05$) in permeate flux between untreated whey permeate processed with 20% air and calcium chloride treated whey permeate without air. In addition, there was significantly higher ($p<0.05$) protein permeation for MF permeates from RW when 20% air was used. These facts suggest that chemical pretreatment of cottage cheese whey could be minimized during WPC production.

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CHAPTER I

INTRODUCTION

Cheese and casein production result in the formation of large volumes of the waste product whey. Whey is characterized as a water-like liquid or serum that separates from the curds during cheese manufacture. Only 10 to 20% of milk is processed into cheese curds, resulting in 80 to 90% whey (Sienkiewicz and Riedel, 1990). Whey is converted into other usable products due to the environmental problems created by its disposal and the growing concern over pollution (Cheyran, 1986).

Whey can be classified as sweet or acid. Sweet whey is the milk serum obtained after the caseins are enzymatically coagulated by rennet at a pH of 5.9 to 6.3. Acid whey is produced when proteins are separated from the milk predominantly by lowering the pH to 4.6. Sweet whey results from production of Cheddar, Swiss, Gouda and Emmental cheeses, while acid whey results from cottage and other fresh cheeses. Whey composition changes according to technological factors and the composition of the milk, which varies with time of year and geographical region. In general, acid whey contains less lactose and a lower proportion of soluble nitrogen than rennet whey, but it has a higher calcium content. (Zadow, 1986; Sienkiewicz and Riedel, 1990). Whey is a liquid mixture of a variety of chemical compounds. Total solids account for approximately 6%, of which 70% or more is lactose and protein is around 12%.

Minerals, vitamins and fat are present, but at lower concentrations (Carić, 1994; Gerberding, 1995). Table 1 shows the compositional breakdown of sweet and acid whey.

Although whey contains about 50% of all milk constituents, the concentrations are very low. Therefore, whey has commonly been considered a waste product rather than a by-product. Nevertheless, whey proteins have a high nutritional value and if extracted under mild conditions will posses useful functional properties (de Wit, 1981; de Wit et al., 1986; de Wit, 1990; Mangino,1992; Morr 1992; Aguilera, 1995). Among the most important whey proteins, also called serum proteins, are β-lactoglobulin, α- lactalbumin, bovine serum albumin and immunoglobulins.

Table 1. Composition of Cheese Whey (Carić, 1994).

Component	Sweet Whey (%)	Acid Whey (%)
Total solids	6.35	6.50
Moisture	93.70	93.50
Fat	0.50	0.04
Protein, total	0.80	0.75
Lactose	4.85	4.90
Ash	0.50	0.80
Lactic acid	0.05	0.40

Many technologies for the production of whey products have been utilized, but more research is needed to make them cost effective. World production of whey is increasing tremendously as a result of an increase in cheese production of about 3% to 4% each year (Cheyran, 1986). According to Gerberding (1995), cheese whey production levels in the United States and in the world reached 25,148 and 121,969 thousand tons, respectively, in 1995.

The advantage of the functional and nutritional characteristics of whey proteins and whey products such as whey protein concentrate (WPC) should be exploited further. Although these products have proven to have a number of applications for the food industry, they are available with a high degree of variability in individual protein composition, functionality and flavor, which limits their use as food ingredients (Sienkiewicz and Riedel, 1990). Table 2 shows how whey solids are added to food products and the particular functional characteristics imparted.

Because whey is a rich source of many compounds it has been studied for many years. Sienkiewicz and Riedel (1990) is a good source of information about the different uses of whey and its technology. Several unit processes, including concentration, drying, demineralization, membrane processing, lactose hydrolysis and fermentation may be applied to all wheys. However, many processes will depend on specific compositional factors to make them commercially feasible. Whey is usually processed into several different powders or concentrates for different uses. According to Morr (1992), these include

Table 2. Use of Whey Solids in Foods (adapted from Carić, 1994).

Product Type	Whey Solids Added (%)	Functional Properties
Baked goods	3.0 (of flour weight)	Flavor, texture, shorter dough time, improved keeping quality
Dry mixes	10.0	Tenderizing, color, flavor
Ice cream	2.7	Flavor, acid, and fruit stability
Sherbet	4.0	Flavor, acid, and fruit stability
Confections	10.0	Flavor, body, moisture retention
Icings, frostings	6.0	Whipping properties
Jams, apple butter	4.0	Flavor
Water ice (on a stick)	2.6	Furnish calcium, phosphorus
Batter mix (for frying)	5.0	Color, flavor
Whey-soy beverage (citrus flavor)	6.0	Lactic acid (from acid whey), flavor
Process cheese	10.0	Body, flavor
Whey-soy blend, dried (2/3 whey, 1/3 soy)	66.7	Masks soy flavor
Whey-soy blends (fat-free soy flour)	40.0	Masks soy flavor, higher in protein

partially or totally dry powders, WPC containing from 35 to 75% protein, and whey protein isolates (WPI) with a protein concentration of at least 90%. The manufacture of WPC involves a series of processing treatments. Cheese whey solutions containing only about 0.5 to 0.7% protein are clarified from fines and pasteurized. Then, it is concentrated by ultrafiltration (UF) to a volume concentration ratio of 20 to 25. The UF retentate is diafiltered against ≥ 3 volumes of purified water to wash away lactose and salts, and finally it is spray dried (Sienkiewicz and Riedel, 1990; Morr, 1992; Huffman, 1996). Several alternative approaches to whey utilization have been proposed, however, there is little information regarding the economics of these processes (Zadow, 1986). Some of the alternative processes for whey processors can be seen in Figure 1.

Ultrafiltration has been increasingly used as a concentration and separation process in a variety of industries where handling sensitive macro molecules, such as proteins, is necessary. There is no heat added and the products are not subject to chemical denaturation when ultra filtered (Pradános et al., 1994). The processing of cheese whey is a good example of the successful application of membrane technology. UF affords a means of simultaneously fractionating, purifying and concentrating the whey while enhancing its utilization and reducing the pollution problem. However, problems related to membrane fouling during UF of cheese whey and protein solutions has captured the interest of many researchers (Hayes et al., 1974; Hanemaaijer, et al., 1989; Labbe et al., 1990; Nilson, 1990; Daufin et al., 1991; Dauffin et al.,

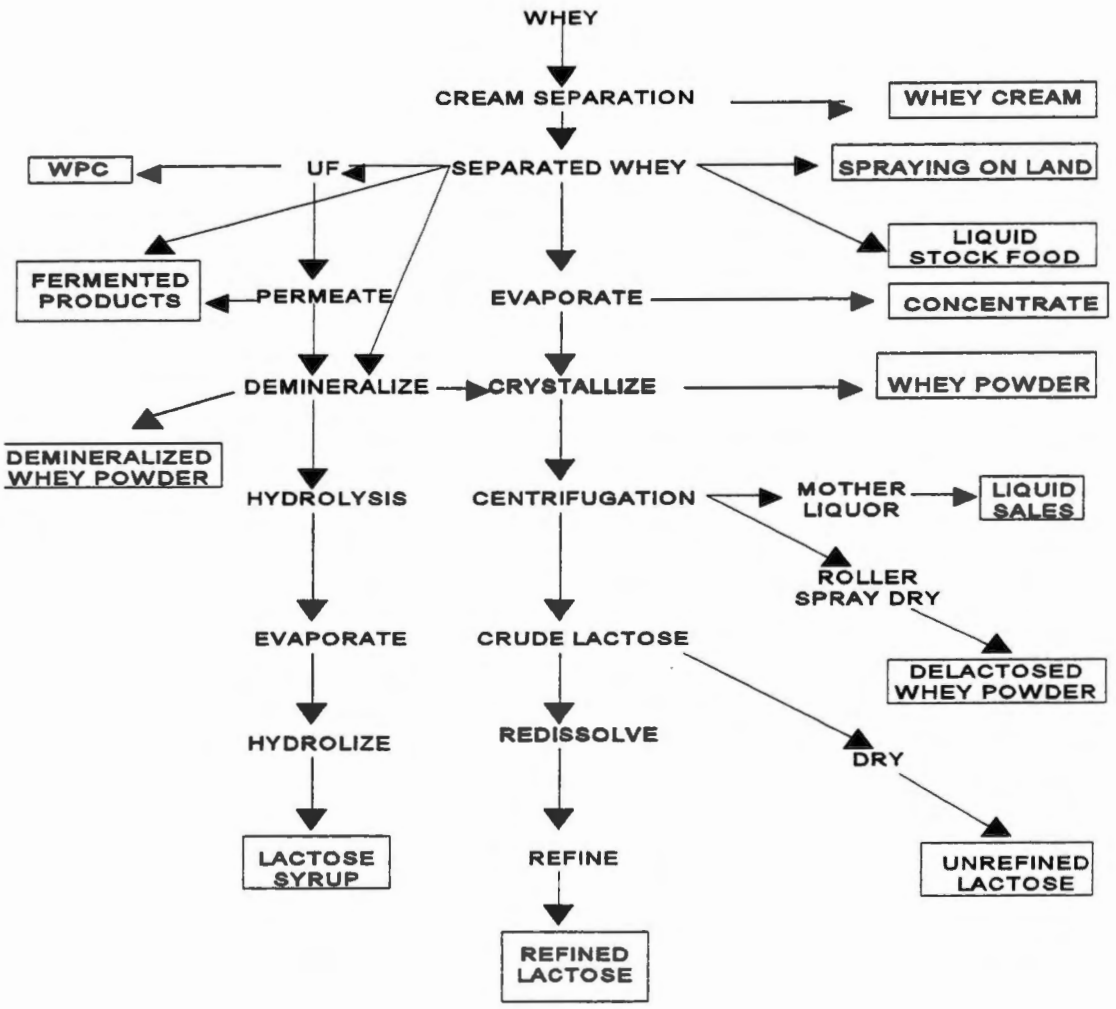

Figure 1. Whey Processing Options.

1993; Marshall et al., 1993; Pradános et al., 1994). Fouling, as a result of specific interaction between the membrane and various solutes in the feed stream, decreases the efficiency of UF. Theories or rules related to the nature of this phenomenon have not been universally accepted.

In the case of whey, minerals have an influence on the fouling of UF membranes. They can interact with the membrane or even precipitate on the membrane causing a reduction of the permeate flux. They contribute to the ionic strength of the solution, affecting protein conformation and dispersion, which is also reflected as membrane fouling. According to Hanemaaijer et al. (1989), the separation characteristics of the membrane are affected by salts with low solubility that precipitate on the membrane and also by adsorption of whey proteins, particularly bovine serum albumin. Both factors affect the pore size distribution of the membrane. Considering fouling by whey salts, both, calcium and calcium-phosphate complex play a major role (Cheyran, 1986; Daufin et al., 1991; Rao et al., 1994a; Rao et al., 1994b). In addition, by using a simulated milk ultra filtrate, a liquid containing the major salts present in whey, Hanemaaijer et al. (1989) showed that other salts, such as Na, Mg and S did not deposit in the membrane and only small amounts of K and Cl were observed under X-ray micro-analysis. Salts in cottage cheese whey adsorb strongly to the membrane. According to Cheyran (1986), increasing the pH of whey will increase the amount of insoluble calcium salts, which will precipitate and increase fouling. Soluble calcium salts can also interact and bind to negatively

charged groups on the membrane causing a "salt-bridge" between whey proteins and the membrane, leading to faster protein fouling.

Residual whey lipids (RWL) also impair UF during WPC manufacture. These lipids include, in general, small milkfat globules and phospholipoprotein complex (PLPC). During membrane separation, the higher the RWL- the lower the resulting flux rate. Residual whey lipids result in WPC with 4 to 6% total lipids, which affect protein functionality and the flavor quality of this product (de Wit et al., 1986; Vaghela and Kilara 1996).

Although several pretreatments have been proposed to remove minerals and lipids to overcome this problem, they have not been universally adopted by the industry. These pretreatment options include chemical pretreatment and/or microfiltration (MF) prior to the UF of whey (Lee and Merson, 1976a; Lee and Merson, 1976b; Kuo and Cheyran, 1983; Maubouis et al., 1987; Morr, 1987; Patocka and Jelen, 1987; Kim et al., 1988; Rinn et al., 1990; Karleskind et al., 1995a).

The fouling effects of UF membranes are characterized by an "irreversible" decline in flux with time, leading to higher cleaning and operating costs. Powerful cleansing agents are needed to restore the flux, but at the same time, they can damage the membrane. According to Kim et al. (1992), fouling during protein UF may be due to surface adsorption/deposition of solute; gradual, irreversible changes to the polarized layer (cake consolidation); and adsorption /deposition of solute within the membrane. Attempts to decrease

fouling during cross-flow microfiltration and ultrafiltration processes have been made by many researchers. Some of these methods include pulsation, back washing and addition of baffles to membranes. Nevertheless, they increase energy requirements. Cui and Wright (1996) and Bellara et al. (1996), have shown the effect of gas-liquid two-phase crossflow to solve the problem of concentration polarization during the UF of protein solutions. According to these researchers, gas sparging disrupts the concentration polarization boundary layer. They have obtained flux enhancements of 10 to 60% for albumin solutions. Air bubbles increase the mass transfer rate due to an increase in the solution velocity. This helps reduce the rate of permeate flux decline by increasing the shear force at the membrane wall.

The advantages of pretreating cheese whey to improve UF membrane performance during WPC manufacture and to produce WPC with improved functional and compositional properties have been well documented (Kim et al., 1989; Harper, 1991; Pearce et al., 1991; Daufin et al., 1994; Karleskind et al., 1995a;). Nevertheless, there is a need to find a method to improve UF performance by increasing permeate flux and reducing chemical pretreatments applied to the whey, especially if this can be achieved with lower energy requirements, by applying air to the system.

The main objective of this research was to improve permeate flux during MF and UF of cottage cheese whey by applying chemical pretreatments and/or two-phase crossflow MF and UF. A secondary objective was to detect

differences in calcium content and protein permeation for the chemically treated and untreated whey, when air was introduced to the microfilter.

CHAPTER II

LITERATURE REVIEW

A. Whey and Whey Protein Chemistry.

Whey is a very diverse and complex mixture of organic and inorganic compounds. Its chemical composition has been widely studied since the early 1900's. It is important to remember that there is considerable variation in the composition of whey. It will vary, according to the source, and seasonal and technological factors. Although the composition of sweet whey has received extensive analysis, less is known about acid whey. One of the reasons is that acid whey has a much lower pH than sweet whey (pH 4.1 compared to pH 5.9-6.7). Thus, it has been utilized to a smaller extent in industry because of a bitter or acidic flavor. Also, as mentioned before, sweet whey has a slightly higher concentration of protein and lactose and a lower amount of ash and water than acid whey.

Whey is also rich in minerals and vitamins. Table 3 shows the mineral analysis of whey powders for acid and rennet whey, while Table 4 gives the average vitamin content of these powders.

Whey proteins are usually defined as those nitrogen compounds that remain in the milk serum after casein has been precipitated at pH 4.6 (de Witt, 1981). They represent about 20% of the proteins present in milk. During cheese

Table 3. Average Mineral Content of Whey Powders.
(adapted from Sienkiewicz and Riedel, 1990)

Minerals	Rennet whey powder	Acid whey powder
Calcium (mg/100g)	878	2,404
Phosphorous (mg/100g)	1,096	1,588
Sodium (mg/100g)	1,287	1,087
Potassium (mg/100g)	1,855	1,915
Magnesium (mg/100g)	178	224
Zinc (mg/100g)	2.10	8.10
Iron (mg/100g)	0.90	1.30
Copper (mg/100g)	0.03	5.30
Iodine (mg/100g)	0.07	8.64

Table 4. Average Vitamin Content of Rennet and Acid Whey Powders.
(adapted from Sienkiewicz and Riedel, 1990)

Vitamin	Rennet acid powder	Acid whey powder
Vitamin A (IU/100g)	136	107
Vitamin C (mg/100g)	1.41	0.33
Vitamin B_6 (mg/100g)	0.59	0.62
Vitamin B_{12} (mg/100g)	2.40	2.50
Tocopherol (mg/100g)	0.06	0.07
Thiamin (mg/100g)	0.51	0.49
Riboflavin (mg/100g)	2.14	1.85
Pantothenic acid (mg/100g)	11.50	11.40
Biotin (µg/100g)	43.00	35.00
Niacin (mg/100g)	1.30	1.16
Folic acid (mg/100g)	0.01	0.03

making, these proteins remain together and unlike casein, continue to be soluble in milk at pH 4.6. For this reason, they have been grouped together representing a characteristic group of globular proteins (de Wit, 1981). The chemical nature and functionality of whey proteins has been an interesting focus of study by many researchers. Although the most important whey proteins are β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins, other proteins such as proteose peptones, traces of various enzymes, of which peroxidase is the most abundant (1% of total whey proteins) and ferro-proteins are also present (Sienkiewicz and Riedel, 1990). β-lactoglobulin is the most important of the whey proteins, found in concentrations of 2 to 3 g/l in milk. The biological function of this protein is not known, and it is absent in human milk. Nevertheless, it represents more than 50% of the total whey protein content in cow milk and the functional properties of WPC are mostly attributed to the properties of this protein. α-lactalbumin is the second most important whey protein, found in concentrations of 0.7 to 1.3 g/l in milk. Both, β-lactoglobulin and α-lactalbumin are synthesized in the mammary gland, while bovine serum albumin and immunoglobulins pass into the milk from the blood. In general, whey proteins differ from caseins in properties and structure. They are not associated into micelles, but are molecularly dissolved and susceptible to heat denaturation. According to de Witt (1981), these proteins may refold again to their three dimensional structure under appropriate conditions, but in many cases they polymerize to aggregates, making the process irreversible. The

major whey proteins, α-lactalbumin and β-lactoglobulin are soluble in aqueous solutions at room temperature, even at their isoelectric points (Pearce, 1983). Table 5 shows a general compositional breakdown of the protein fraction in whey, with their respective molecular weights. β-lactoglobulin consists of 162 amino acid residues, with two internal disulfide bonds and one free thiol group. Naturally, between pH 5.2 and 6.7 this protein exists as a noncovalently linked dimer, with a molecular weight of 36,700 D. Near the isoelectric region (pH 3.5 to 5.2), these dimers are associated to form octamers, reaching a molecular weight of 140,000 D. Below pH 3.5 these octamers dissociate to monomers (Knopp, 1992; Goff and Hill, 1993). It is considered one of the most stable of the whey proteins with a denaturation temperature of approximately 78°C. The denaturation temperature of this protein is pH dependent (Robin et al., 1993).

α-lactalbumin contains 123 amino acid residues, eight cysteine groups and four disulfide bonds (Goff and Hill, 1993). It is present in all milk where the presence of lactose can be demonstrated, and it is involved in the last stages of lactose synthesis. (Sienkiewicz and Riedel, 1990). It is considered the least stable of whey proteins, with a denaturation temperature of 62°C. Nevertheless, this protein requires more heat per gram for unfolding. According to Robin et al. (1993), the reversible heat denaturation of α-lactalbumin at pH 6 is due to dissociation and reassociation of calcium ions from the protein. Thus, unlike most proteins, this protein has the unique property of being stable in the presence of calcium ions. Minimum solubility is obtained at pH 4.2, which

Table 5. Whey Protein Composition.
(adapted from Gerbending, 1995 and de Witt, 1981)

Protein	% of Total Whey Protein	Mol. Wt. (Daltons)
β-Lactoglobulin	56.5	18,277
α-Lactalbumin	19.4	14,175
Bovine Serum Albumin	7.1	66,267
Immunoglobulins	13.4	
IgA	0.5	300,000-500,000
IgG	12.4	150,000-170,000
IgM	0.5	900,000-1,000,000
Proteose-peptones	3.6	4,100-22,000

corresponds to the isoelectric point. Bovine serum albumin (BSA) is a complex molecule that consists of 582 amino acid residues with 17 intramolecular disulfide bonds and an isoelectric point of 4.9 to 5.1. It has very similar physiological and immunological characteristics to the albumin found in human plasma. BSA accounts for about 6% of whey proteins, depending on the stage of lactation of the animal, and it is believed to help in the transport of fatty acids and ions (Gerbending, 1995). A greater tendency toward hydrophobic aggregation of this protein has been found at temperatures above 40°C, because over this temperature, its hydrophobic residues are exposed (Knopp, 1992).

Immunoglobulins are a family of large molecular weight proteins, biologically known as antibodies. Four classes of immunoglobulins are found in

bovine milk: IgG, IgA, IgM and IgE, with IgG being the principal class. All of them are made of polymer or monomer units consisting of four polypeptide chains as a basic unit (Gerberding, 1995).

The proteose peptone fraction is a mixture of several phosphorous glycoproteins which contain approximately 10% carbohydrates. These molecules are thought to be derived from casein, and they are very stable at high temperatures (95 to 100°C) and under acidic conditions (pH 4.6 to 4.8) (Sienkiewicz and Riedel, 1990). The function of this protein fraction is not clear, neither is its effect on the functionally of WPC (Gerberding, 1995).

B. Functionality of Whey Proteins.

Whey protein concentrates can be used as ingredients in a variety of formulated products since they are Generally Recognized As Safe (GRAS) and because of their various functional properties (Morr and Foegeding, 1990; Sienkiewicz and Riedel, 1990; Mangino, 1992; Morr, 1992; Aguilera, 1995; Huffman, 1996). However, there is no WPC that possesses all these functional properties.

1. Nutritive Value.

Whey proteins are used as an excellent source of essential amino acids

that are easily digested. The overall nutritional value of some foods can be fortified by adding these proteins, balancing certain deficits, like low levels of lysine in wheat flour and rice, and low levels of methionine in soy. Whey proteins also contain high levels of leucine, isoleucine and valine, which are of interest for sports drinks and nutrition bars. They can be used as a source of nutrients in fortified fruit juices, and diet or health foods (Huffman, 1996). The amino acid composition of acid and rennet whey is shown in Table 6.

2. Solubility.

Solubility is an important functional property since it influences foaming and emulsion formation. Whey proteins that have not been denatured by heat posses good solubility over a wide range of pH. According to Huffman (1996), more than 80% of these proteins remain soluble after heating an aqueous solution of WPC at 90°C for 5 minutes. The solubility of whey proteins in heated products can be increased by addition of sugar, which improves their heat stability. The acid solubility of whey proteins makes them especially important in applications such as salad dressings and acid beverages. Also, WPCs obtained by UF are usually soluble up to 90% in the pH range of 3 to 8 (Sienkiewicz and Riedel, 1990).

Table 6. Amino Acid Composition of Wheys.
(Sienkiewicz and Riedel, 1990)

Amino acid	Acid whey (mg/100g)	Rennet whey (mg/100g)
Lysine	72.3	71.6
Histidine	15.5	13.1
Arginine	18.6	18.1
Aspartic acid	80.0	81.8
Threonine	40.7	50.2
Serine	37.7	40.8
Glutaminic acid	139.1	140.1
Proline	40.8	48.8
Glycine	16.2	16.8
Alanine	33.2	37.1
Cysteine	15.4	9.6
Valine	43.1	46.2
Methionine	14.9	13.8
Isoleucine	38.8	49.8
Leucine	85.1	81.1
Tyrosine	23.2	19.0
Phenylalanine	27.1	24.5
Tryptophane	15.5	16.3

3. Water-binding capacity.

The water binding capacity of whey proteins is very low compared to soya protein. Native and denatured whey proteins have capacities of 0.5 to 1.2 grams of water per gram of dry matter. WPC obtained from acid whey has a stronger water-binding capacity than those obtained from rennet whey. Heating of whey proteins increases the water-holding capacity. As the protein partially unfolds, additional water-binding sites are exposed. This functional property has been investigated for applications in meat and gelled products (Sienkiewicz and Riedel, 1990; Huffman, 1996).

4. Viscosity.

The viscosity of WPCs is affected by several factors, such as amount of dry matter, protein content of the solutions, pH, temperature, ionic strength, and size and shape of the molecules. Since these proteins are much smaller than casein micelles, this is reflected as a lower viscosity of WPC solutions (less than 5 cp at 10% total solids). However, this low viscosity can be increased by heating whey proteins. The partial unfolding increases the volume occupied by the protein. Further protein aggregates can increase this volume even more, thus increasing viscosity. The ability of WPCs to increase viscosity is the basis for its use as a thickener in soups, sauces and yoghurt (Sienkiewicz and Riedel,

1990; Huffman, 1996).

5. Gel Formation.

WPCs and WPIs in concentrations of 80 to 90% have important applications in food systems because of their tendency to form irreversible heat-induced gels. This gel formation depends on factors such as composition, extent of denaturation, concentration, pH, temperature and ionic strength, among others (Sienkiewicz and Riedel, 1990; Aguilera, 1995). Whey proteins start to form gels after heating at 65°C., giving gels of different characteristics (curdy or smooth, shiny, strong and elastic like egg white) (Huffman, 1996). The major gelling proteins in whey are β-lactoglobulin and BSA. However, since β-lactoglobulin is present at much greater concentrations (10 to 20 times higher) it is primarily responsible for this functional property (Aguilera, 1995). The gel formation of WPCs has been a topic of research for many years since this can be used to modify the textural characteristic of food systems (hardness, elasticity and cohesiveness), with applications in meat, cakes and seafood products. The gel matrix entraps water, increasing the water-holding capacity. If the gel is strong, it will hold water and prevent syneresis or moisture loss, improving the yield of products such as hams and surimi (Huffman, 1996).

6. Emulsification.

Whey proteins can act as emulsifiers since they have both, hydrophobic and hydrophilic regions. This capacity is affected by the protein size, shape and solubility, and the protein concentration of the solution, ionic strength and finally, the conditions under which the WPC was dried (Sienkiewicz and Riedel, 1990). Although WPCs have a lower emulsifying capacity than sodium caseinate, due to a more regular sequence of hydrophobic and hydrophilic groups and also due to a more compact formation (Sienkiewicz and Riedel, 1990), the fact that whey proteins have fairly good solubility under acidic conditions, helps WPCs to be utilized in applications as emulsifiers in systems like salad dressings (Huffman, 1996).

7. Foaming.

The ability of whey protein concentrates to form foams is probably one of the most studied properties, because of the continuous search for egg replacers possessing the same functional characteristics. The extent of the protein interaction with the air-water interface is affected by the ability of the molecule to reach the interface and unfold to form viscous films (Phillips et al., 1995). The speed of air incorporation and the foam stability is also affected by factors like degree of denaturation, residual whey lipids and phospholipid content, calcium

content, protein and carbohydrate concentrations, pH and processing equipment (Sienkiewicz and Riedel, 1990; Huffman, 1996). The maximum foam stability and overrun in defatted whey proteins is reached at a pH of 4.6 to 5.0 (in the isoelectric range). This characteristic can be desirable or undesirable in specific food applications. It is important for aerated frozen desserts, cakes, confections, whipped toppings and meringues but not desirable for fortified fruit juices. (Huffman, 1996).

Morr and Foegeding (1990), agree that WPCs and WPIs are the best forms to use whey proteins due to their wide range of application in the food industry. Nevertheless, they propose that their manufacturing processes should be standardized to increase the acceptance of these products in the market. According to Morr (1992), there are variations in overall composition, individual protein composition, functionality and flavor of the commercially available products, which limits their acceptance as food ingredients.

C. Membrane Separation Processes Available for Whey Proteins.

The recovery of whey proteins has presented a challenge to the dairy industry for a long time. Before membrane separation processes were available, whey proteins were isolated using precipitation techniques. Today, thanks to the development of several membrane separation processes, the production of technologically functional WPCs with different protein contents are possible

(Sienkiewicz and Riedel, 1990). Since the ultimate goal is to maintain the protein functionality and solubility, methods such as ultrafiltration and microfiltration have found commercial success.

Ultrafiltration is a widely used method for fractionating whey proteins, even though it has some drawbacks, such as high capital and operating costs, problems with fouling of the membrane and regular in-place cleaning and sanitation of the membrane to minimize microbial contamination (Gerberding, 1995). However, recent improvements in ultrafiltration equipment and the availability of a variety of membrane materials have contributed to its acceptance by dairy processors. In addition, one of the advantages of this process is the flexibility in producing WPCs with the desired protein content (Hanemaaijer, 1985) (Table 7).

According to Sienkiewicz and Riedel, 'ultrafiltration is a membrane separation process which fractionates the individual solutes, as a function of their size and structure, by means of a pressure gradient and a semi-permeable membrane.' Ultrafiltration retains macromolecules larger than 10 to 200 Å, or 0.001 to 0.02 µm (Cheyran, 1986). Usually, ultrafiltration is applied when the molecular weight cut-off (MWCO) value of the membrane is above 500, usually in the range of 1,000 to 500,000 daltons (Sienkiewicz and Riedel, 1990, Mannapperuma, 1997). This allows the separation and concentration of large macromolecules, like whey proteins in the retentate leaving a permeate fraction

Table 7. Composition of WPCs after Ultrafiltration (%).
(adapted from Sienkiewicz and Riedel, 1990).

Constituent	Protein Content in %			
	35	50	60	80
Water	4.6	4.3	4.2	4.0
Crude protein (N x 6.38)	36.2	52.1	63.0	81.0
Lactose	46.5	30.9	21.1	3.5
Fat	2.1	3.7	5.6	7.2
Ash	7.8	6.4	3.9	3.1
Lactic acid	2.8	2.6	2.2	1.2

which contains mainly water, lactose and salts. Large scale ultrafiltration plants consume about 0.014 KWh per Kg of treated whey (Sienkiewicz and Riedel, 1990).

Membrane materials for ultrafiltration are normally polymers or a combination of ceramic and metal. The former type include sulfonated polysulfone, polysulfone, polyethersulfone, polyvinilidenefluoride, and polyacrylonitrile membranes, among others. The ceramic type include zirconia/alumina membranes. All these membranes are assembled in different module configurations, such as flat plate, tubular, hollow fiber and spiral. A schematic of the hollow fiber membrane used in this research is shown in Figure 2.

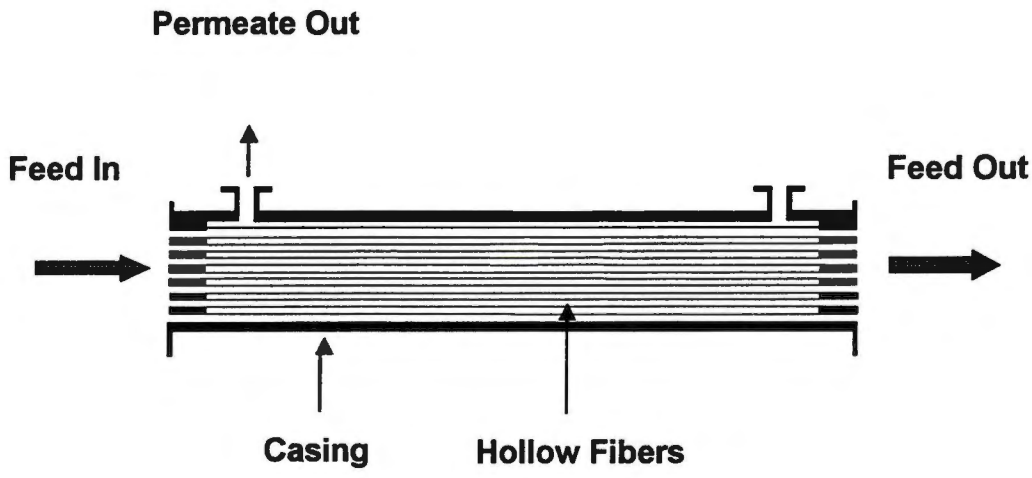

Figure 2. Hollow Fibers Membrane.

These modules can withstand the pressures required in filtration and also the cross-flow velocities needed to maintain a clean membrane (Mannapperuma, 1997).

The ability to produce large volumes of filtrate in a relatively short period of time is a measure of the performance of a membrane filtration system. Permeate flux or 'J' is defined as the volume of permeate that flows through a unit area of membrane in a unit period of time. It is one of the parameters used universally to measure performance. The typical units are liters per square meter per hour (lmh) or gallons per square foot per day (gfd) (Cheyran, 1986, Mannapperuma, 1997). Another of the parameters used to measure performance of the membrane is the degree of purity of the filtrate with respect to the solute concentration, sometimes called 'solute rejection.'

Unlike conventional 'dead-end' filtration, where particles retained by the filter build up with time, resulting in increased resistance to filtration, cross-flow ultrafiltration allows a continuous separation of the solids from the feed stream. Relatively high fluxes can be maintained over longer periods of time, because "the bulk phase under pressure is forced to flow along the surface of the membrane, sweeping the retained particles so that the cake layer remains thin and the resistance to filtration remains low" (Mannapperuma, 1997).

On the other hand, another membrane separation process, microfiltration, is relatively new to whey processing. This is the coarsest of the membrane filtration techniques. It requires lower operating pressures than UF, usually

between 10 and 15 psi. These membranes are classified by pore diameter cut off, usually in the range of 0.1 to 10 μm. (Mannapperuma, 1997), separating larger particles, such as microorganisms, casein fines, phospholipoprotein particles and fat globules from the whey (Jelen, 1991). By applying MF prior to UF, an almost sterile product can be obtained. In addition, low fat contents in the WPCs can have many advantages. A positive feature is that WPCs obtained after applying this process show good whippability and foam stability, due to the reduction in the fat content (Sienkiewicz and Riedel, 1990). The use of microfiltration as a method of pretreating whey prior to UF has been investigated in several papers and will be discussed later (Hanemaaijer, 1985; Maubois et al., 1987; Maubois, 1988; Rinn et al., 1990; Pearce et al., 1991; Karleskind et al., 1995a; Karleskind et al., 1995b).

Polymer and ceramic/metalic membrane materials are the most common, including polysulfone, polyethersulfone, polyester, polypropylene among polymers and alumina, zirconia/alumina, zirconia/metal, zirconia/carbon among ceramic/metalic ones (Mannapperuma, 1997).

Several other techniques are currently available for processing whey, which can be applied in combination with other processes. Different products can be obtained from whey, and it is impossible to discuss all of them here. Reverse osmosis is also a pressure driven process that uses semipermeable membranes. It is used in conjunction with UF or gel filtration for the concentration and partial demineralization of whole whey and for concentration of

permeates from the UF units. Other methods include gel filtration, ion exchange chromatography, ion exchange adsorption, and electrodialysis (Sienkiewicz and Riedel, 1990). Table 8 summarizes some of these processes with their advantages.

D. Fouling of Ultrafiltration and Microfiltration Membranes.

The term fouling is referred as a gradual decline in flux with time when operating parameters, such as pressure, temperature, velocity, concentration, etc., are kept constant (Cheyran, 1986; Mannapperuma, 1997). This phenomenon has been a challenge in membrane applications for a long time and has led to many studies. Whey and its derivatives have often been considered a model system, since it is the main food fluid enhanced by UF and also because its components are representative of other fluids in the biotechnology and food industry. Besides the non-fouling lactose, whey proteins and calcium salts are generally identified as severe foulants (Cheyran, 1986; Hanemmaaijer et al., 1989; Rao et al., 1994b).

According to Marshall et al. (1993), a difference must be made between membrane fouling and concentration polarisation. Concentration polarisation is independent of the physical properties of the membrane, but a function of hydrodynamic conditions in the membrane system. This is considered to be the development of a concentration gradient of the retained particles near the

Table 8. Characteristics of Separation Techniques used in Whey Protein Recovery (adapted from Sienkiewicz and Riedel, 1990).

Technique	Advantages	Disadvantages
Ultrafiltration	Fractionation with concentration. Low production costs.	Membrane fouling Process temperature and pH limited by the membrane.
Reverse Osmosis	Concentration of all constituents. Low production costs.	No fractionation. Maximum concentration of 22%.
Microfiltration	Separation of microorganisms, casein fines and fat.	No concentration of proteins. Initial cream separation required. Process temperature and pH limited by the membrane. Membrane fouling.
Gel Filtration	Complete separation of low molecular weight constituents in one stage. Long life span of gel.	Pre and after concentration required.
Ion Exchange	Removal of salts	Regeneration of resins necessary. No concentration of proteins.

membrane, that forms a layer on the membrane surface during the first minutes of operation (Figure 3). It is a reversible process, that can be overcome by increasing the velocity, deceasing the pressure or decreasing the concentration. Therefore, it is not considered a form of fouling (Mannapperuma, 1997). Nevertheless, irreversible changes over time can be produced as a consequence of concentration polarisation. This form of fouling occurs on the surface of the membrane, where solutes accumulate. The mechanism of this fouling-layer formation is governed by membrane-solute and solute-solute interaction. As stated before, proteins are common surface foulants in UF. Inorganic compounds such as calcium, calcium phosphate also precipitate on the membrane (Cheyran, 1986; Marshall et al., 1993).

On the other hand, another form of fouling is the deposition of components from the feed stream on the membrane surface or its pores. In this case, there is a change in the membrane behavior. Pore plugging occurs with solutes that are small enough to enter and get trapped in the pores. Solute adsorption is another form of fouling, recognized as an important mechanism in protein UF (Nilson, 1990; Kim et al., 1992; Mannapperuma, 1997). In addition, fouling of biological feed streams, like whey can also be due to fats and bacteria (Marshall et al., 1993).

Lee and Merson (1976a), examined proteins from chemically treated cottage cheese whey and observed membrane fouling deposits by scanning electron microscopy. Depending upon the treatment, different forms of deposits

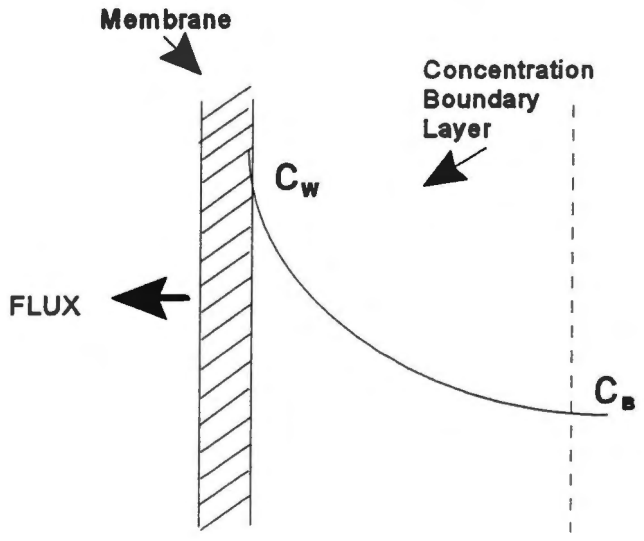

Figure 3. Schematic Representation of Concentration Polarisation and the Concentration Profile during UF. (where C_W = conc. at the membrane wall and C_B = Conc. at the boundary layer) (Cheyran, 1986)

were found. Fouling was reduced when the formation of protein sheets over the membrane was retarded. These protein layers of 0.5 to 1.0 μm thickness, obtained after 35 minutes of processing were referred to by the authors as a multilayer adsorption. Suki et al. (1984) examined the relationship between flux decline and proteins deposited or bound to UF membranes. They used solutions of BSA (≥97% pure), over a wide range of pH, with or without salt, for cross-flow experiments lasting 5 hours. The authors also concluded that the fouling phenomenon involves multi-layers. According to them, the bulk concentration had an influence on the rate of deposition, but the ultimate values of mass deposited depended on other factors, such as membrane type, solution pH, salt content and system hydrodynamics. When the membrane skin is in contact with a protein solution, a significant increase in the hydraulic resistance of the membrane can occur, due to the interaction between the membrane and the proteins (Hanemaaijer et al., 1989).

Several attempts have been made to develop mathematical models for this flux decline during UF (Suki et al., 1984; Daufin et al., 1992; Koutake and Matsuno, 1993), and to determine which protein is the major contributor to this phenomenon. According to Merin and Cheyran (1980), α-lactalbumin causes the greater flux decline during the initial periods of UF, but long term, β-lactoglobulin is the major contributor to fouling. After X-ray analysis of the membrane surface, they also found that various salts in cheese whey (chlorides, calcium phosphates, potassium and magnesium) are adsorbed or trapped in the pores of the

membrane. The authors suggest that total or partial removal of whey salts would considerably improve flux. In another study made by Tong et al. (1989), a proteinaceous fraction of membrane foulants was characterized and analyzed by sodium dodecylsulfate polyacrylamide electrophoresis (SDS-PAGE), after using a 10 kD MWCO polysulfone membrane for whey UF. The foulants consisted of 13.5 to 22 kD peptides adsorbed to the membrane, which were identified as casein proteolysis products, present only when calf rennet is used for cheese manufacture. Another study made by these authors had also suggested that permeate flux during UF depended on the type of milk coagulant used in the cheese making process (Tong et al., 1988). However, besides these peptides, α-lactalbumin was the main protein adsorbed to polysulfone membranes, which agrees with previous studies made with UF of cottage cheese whey (Tong et al., 1989).

Kim et al. (1992), examined deposits formed during UF of 0.1% solutions of BSA using various UF membranes. They concluded that the fouling mechanism depends on the type of membrane used. In addition, they found aggregates and cakes (sheets of proteins) deposited on the surface of the membrane using a high resolution field emission scanning electron microscope. Higher initial UF flux promoted the formation of these aggregates, initiated by supersaturation of proteins near the pores, due to high convective flows, while a lower initial UF flux showed cake formation.

Although there are strong discrepancies related to the fouling mechanism

in UF and MF, there is no doubt that it is a complex phenomenon, where surface and internal fouling occur at the same time to a greater or lesser extent. This mechanism is strongly influenced by experimental conditions, operating conditions, and the properties of the feed and of the membrane. Figure 4 shows the various stages of flux decline as explained by Marshall et al. (1993). According to the authors, three separate phases of flux decline can be observed. The rapid decline in flux during the first minute of operation can be due primarily to concentration polarisation. Flux continues to decline for about one hour, due to protein deposition. Initially this deposition is a monolayer adsorption that eventually builds up to a complete surface layer. Finally, the system reaches a quasi-steady-state, where further deposition of particles or cake consolidation takes place. Here, flux declines slowly.

Although the pore size in microfiltration is considerably larger than the proteins, severe pore plugging by these macromolecules can also occur. The advantage of high protein retention in UF becomes a disadvantage in some MF applications, where protein transmission is required. Researchers have investigated the deposition of BSA on MF membrane, concluding that it occurs in two phases. A rapid deposition in the first phase, is the result of a strongly bound monolayer adsorption onto the surface of the membrane. A second and slower phase is the build up of multilayers of proteins that are weakly bound, with further pore plugging, due to increased collisions (Marshall et al., 1993). A study of fouling of inorganic membranes during UF of different feed streams was made

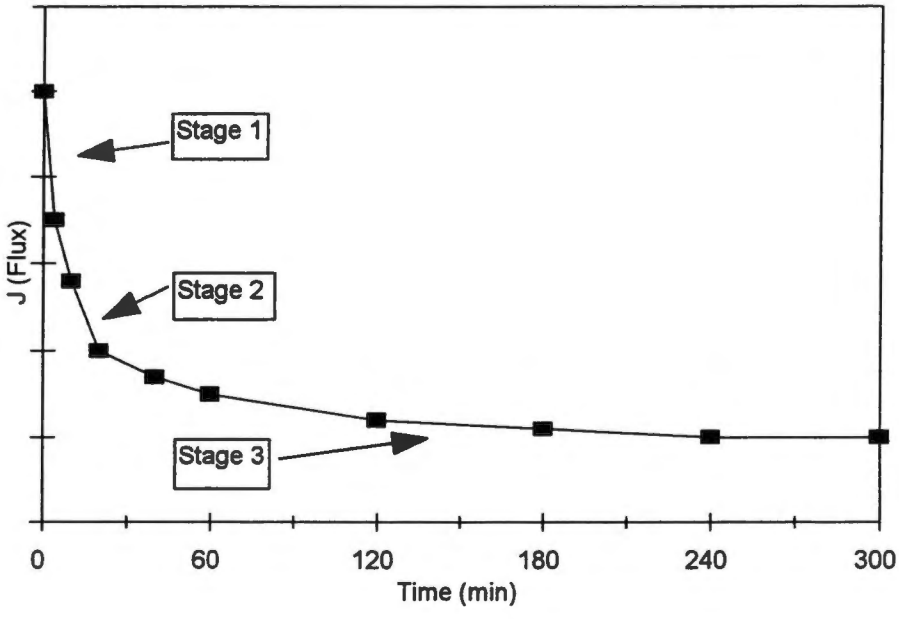

Figure 4. Stages of Flux Decline (adapted from Marshall et al., 1993).

Stage 1 - Flux loss due to concentration polarisation.
Stage 2 - Flux loss due to protein deposition.
Stage 3 - Flux loss due to particle deposition or consolidation of the fouling material.

by Daufin et al. (1991). They showed that despite the absence of lipids in whey microfiltrates, which lead to an increase in UF fluxes, microfiltration did not solve the problem of irreversible fouling, caused by a mixture of calcium phosphates.

Many attempts have been made to decrease the fouling problem during UF and MF of whey and proteins solutions. Processing variables such as transmembrane pressure, feed concentration, temperature and cross-flow velocity can be optimized to reduce the amount of flux decline. In general, increasing the feed concentration during UF results in a decrease in permeate flux. When internal membrane fouling dominates, increasing the concentration has a detrimental effect and cake or surface fouling is likely to dominate (Cheyran, 1986; Marshall et al., 1993). Increasing the transmembrane pressure greater than 4 bar, initially results in increased permeate flux in UF, but also in a higher fouling rate. Later, the increased rate of fouling leads to a higher component retention. When fouling layers form, they become compressed under high pressures, causing a lower flux (Cheyran, 1986). According to Marshall et al. (1993), "there is an optimum pressure, below which the driving force is too low and above which increased fouling causes a large reduction of flux". On the other hand, increasing cross-flow velocity improves permeate flux in UF and MF, reducing membrane fouling. Generally an increase in the temperature will also increase the permeate flux, due to a reduction in the viscosity and an increase in diffusivity, which helps dispersion of the polarised layer in UF and MF (Marshall et al., 1993). Nevertheless, operating conditions need to be optimized for each

situation.

Pretreatment of the feed material can also have a positive effect on permeate flux. Several treatments have been proposed for the whey system to prevent fouling. However, as mentioned before, they have not been completely accepted by industry.

E. Effect of Whey Pretreatment on Permeate Flux, Fouling and WPC.

Many pretreatments can be applied to whey to improve UF performance. In general, these include pH adjustments, modifications of the mineral content, pH adjustment followed by heat treatment, removal of residual whey lipids (RWL) and microfiltration. However, due to differences in whey composition and operating parameters, it is difficult to standardize these pretreatments for the dairy industry. Sienkiewicz and Riedel (1990), reported the benefits of calcium sequestration for acid whey, using EDTA or citric acid for cottage cheese whey. For rennet whey, they suggest microfiltration using a 1.2 μm pore size membrane, to enable bacteria and fat separation. Adjusting the pH is the simplest way to minimize fouling. Even though macromolecules play an important role, the presence of microconstituents such as salts can be equally detrimental. Kuo and Cheyran (1983), reported significant improvements in permeate flux for treated versus untreated cottage cheese whey, after lowering the pH to 2 or 3, followed by centrifugal clarification. Using a polysulfone UF

membrane with a MWCO of 20,000 D, they also found that 50°C was a more beneficial operating temperature than 40°C or 30°C. Their results reflect the fact that the solubility of whey salts (particularly calcium phosphate) is improved, becoming more likely to permeate through the membrane. Lee and Merson (1976a), obtained increases in UF rates by treating cottage cheese whey with calcium sequestering agents (EDTA), modifying protein side chains or increasing the ionic strength. Since BSA and β-lactoglobulin are sheet forming proteins in whey, by blocking carboxyl and sulfhydryl groups, dispersion of these proteins was improved, thus, retarding the formation of the fouling layers. Acidification of whey also promoted changes in the state of β-lactoglobulin, which at pH below 3.5 dissociates to the monomer form. They concluded that addition of NEM (N-ethylmaleimide), used to block sulfhydryl groups in the proteins, reduced significantly the amount of fouling. However, only addition of EDTA is feasible from a commercial point of view, since NEM is not an acceptable food additive. Patocka and Jelen (1987), reported a 20% to 24% increase in flux by adjusting the pH of cottage cheese whey to 2.5 and 1.5, respectively. Calcium permeation rates were also improved by lowering the pH. Nevertheless, such low pH caused changes in the odor of whey, which makes this procedure questionable for industrial application. The highest flux improvements were obtained when the amount of EDTA used was equivalent to the amount of free calcium in whey. Mehra and Donnelly (1993), also reported an improvement of filtration rate by addition of EDTA, however, this treatment had no effect on the protein

permeation of several whey solutions. Recently, Rao et al. (1994a), studied the fouling characteristics of permeates from different types of milk and whey. They found that calcium rejection values were low for acid and sweet whey in comparison with the milk systems (where about 2/3 of calcium is bound to protein in the form of micellar calcium phosphate). Thus, not all soluble calcium was permeating during UF. They suggested that soluble calcium could also be involved in the fouling mechanism (Table 9). In addition, "further processes which increased the levels of soluble calcium reduced flux and increased fouling."

Table 9. Total Calcium Content (mM) and Calcium Rejection* in some Milk Products (Rejection values in parenthesis).

	Whole milk	Skimmed milk	Buttermilk	Sweet whey	Acid whey
Product	25.5	26.3	20.0	10.5	20.0
Permeate 1[a]	3.1 (0.87)	2.9 (0.88)	4.6 (0.77)	6.8 (0.34)	14.5 (0.27)
Permeate 2[b]	4.7 (0.81)	4.2 (0.83)	4.4 (0.77)	8.2 (0.21)	4.4 (0.11)

[a] Permeate after 10 min of operation.
[b] Permeate after 50 min of operation.
* Rejection (R) = $\frac{\text{Concentration in feed - Concentration in permeate}}{\text{Concentration in feed}}$

Maubois et al. (1987) proposed a different chemical pretreatment in combination with a physical pretreatment (microfiltration), applicable to all wheys and to UF whey retentates. The reason for this modification was the fact that functional properties of WPCs (especially the foaming ability) are known to be negatively affected by residual whey lipids. The procedure involved cooling whey to 2°C, adjustment of calcium content to 1.2 g/Kg with a solution of calcium chloride, pH adjustment to 7.3 with NaOH and a rapid increase in the temperature to 50°C, which was maintained for about 8 minutes. This induced the formation of a white precipitate that slowly separated by sedimentation. Further MF led to a complete removal of this fraction (PLPC). Calcium content was reduced by approximately 60% due to precipitation with lipoproteins, P content was reduced by 70% and Nitrogen by 11% (from lipoprotein removal). Lee and Merson (1976b) had previously stated the benefits of pre-filtering cottage cheese whey before UF processes, but they only proposed the use of large-pore UF membranes as a pre-treatment. Maubois et al. (1987) reported that the cost of MF equipment could be balanced by reduced investments in UF. According to the authors, removal of lipoproteins, which are a significant source of irreversible fouling of UF membranes, results in an increase in UF flux. In addition, the "combined utilization of thermocalcic aggregative properties of the lipoproteins contained in the residual fat and the use of the MF technology", leads to the production of defatted and sterilized WPCs through UF (Maubois, 1988). Later studies made by Kim et al. (1989), using blue cheese whey and centrifugation

instead of MF showed that pretreated WPC proteins were less functional for emulsification than untreated WPC proteins. In addition, the authors could not obtain the same reduction of lipoprotein content reported by Maubois et al. (1987). Lehmann and Wasen (1990), patented a process for dephospholipidating whey in which they modified the procedure proposed by Maubois et al. (1987). In addition to that, the aggregated lipoproteins were separated by centrifugation instead of MF. Karleskind et al. (1995a) obtained WPCs from Swiss cheese whey with ≤0.5% lipids by using chemical pretreatment, MF and UF. They found that isoelectric point precipitation was more effective for lipid removal than thermocalcic aggregation, proposed by Maubois et al. (1987). In addition, significant differences between protein permeation were found for the two MF membranes used. The 0.45μm membrane provided higher β-lactoglobulin recovery than the 0.1μm membrane, while α-lactalbumin was best recovered by the 0.1 μm MF membrane. The authors suggest that further study is needed to optimize the MF process. Knopp (1992), had previously shown that although MF effectively removed RWL from sweet whey, incomplete recovery of proteins was obtained using a 0.45 μm MF membrane. Here, size exclusion HPLC analysis indicated that the permeation ratio was ≤0.538 for α-lactalbumin and ≤0.238 for β-lactoglobulin.

Rinn et al. (1990) evaluated nine different whey pretreatment modifications for producing WPC. After thermocalcic aggregation of the lipoproteins, pretreatment modifications 5 and 6 provided the highest UF flux rate (table 10)

and lowest lipid and phospholipid content. WPCs obtained from these pretreatments also showed the highest functional properties (solubility, foam expansion, gelation and emulsifying activity).

Whey pretreatments have been suggested for over 15 years in order to improve membrane permeability. Still, UF of defatted whey presents problems related to membrane fouling by proteins and calcium phosphate (Labbé et al., 1990; Daufin et al., 1991). Daufin et al. (1993) improved membrane performance of defatted sweet whey. They showed that membrane fouling was reduced when pH was kept constant during the pretreatment. In this way, low levels of calcium and phosphate were maintained in the microfiltrate, which resulted in less protein fouling.

Another approach to solve the fouling effect of protein solutions is shown in the work of Cui and Wright (1996) and Bellara et al. (1996). Although their research has been done only in model systems, significant improvements in UF flux have been achieved. Using gas-liquid two phase crossflow UF (by injection of air to the UF system), Cui and Wright (1996) observed flux enhancements of 320% for dextran solutions, when using two-phase crossflow UF. This increase in flux was more important when concentration polarisation was more severe. According to the authors, air bubbles disrupt the concentration polarisation layer. In addition, gas sparging was more effective in the liquid laminar flow region and promoted the transition to a turbulent flow. Bellara et al. (1996) applied two-phase crossflow filtration to dextran and albumin solutions. Using a hollow fiber

Table 10. Pretreatment Modification and Effect on Flux.
(adapted from Rinn et al., 1990)

Pretreatment modification	Gravity settle	Centrifugal clarification	Microfiltration	UF flux* (mL/m^2 min)
1	no	no	no	3.36 ± 0.04^{F}
2	no	single pass	1.0 µm	4.40 ± 0.04^{B}
3	no	single pass	no	4.31 ± 0.01^{C}
4	yes	no	no	3.44 ± 0.01^{F}
5	no	single pass	0.6 µm	6.21 ± 0.01^{A}
6	no	no	0.6 µm	6.22 ± 0.01^{A}
7	no	20 min recycle	0.6 µm	3.55 ± 0.04^{E}
8	no	30 min recycle	0.6 µm	4.34 ± 0.04^{C}
9**	no	single pass	0.6 µm	4.19 ± 0.01^{D}

* Means of duplicate determinations obtained afted 1, 15, 30 and 45 min of UF.
** pH adjusted to 4.0 before UF.
Values with the same letter group are not significantly different ($p \leq 0.05$).

membrane, they obtained up to 60% increase in permeate flux for the albumin solution. They reported that the mechanism involved in the flux enhancement effect of the air bubbles was physical displacement of the concentration polarisation layer.

CHAPTER III

MATERIALS AND METHODS

A. Whey.

Raw cottage cheese whey (unclarified) was provided by Purity® Dairy (Nashville, TN). Whey was obtained on two dates immediately after cheese manufacture, and transported to the Department of Food Science and Technology at the University of Tennessee, Knoxville, on the same day. Each batch was transferred to clean buckets filled to approximately 10 to 15 L. To minimize source and seasonal variation as well as contamination, the buckets were stored in a freezer at -10°F until ready to process. Average composition of the cottage cheese whey is shown in Table 11.

B. Chemical Pretreatments.

The whey was thawed overnight at 20 to 25°C before processing. Two chemical pretreatments were applied. The first consisted of addition of 0.001M EDTA (Sigma Chemicals, St. Louis, MO) to 10 L of whey. The pH of the whey, which was initially 4.4 to 4.6 was adjusted to 4.1 using 6N HCl and the treated whey was then microfiltered. The second pretreatment consisted of cooling the whey to 2 - 5°C, adding 1.2 g of $CaCl_2$ (Sigma Chemicals, St. Louis, MO) per

liter of whey, adjusting the pH to 7.3 with 6N NaOH and heating to 55°C for 8 minutes by suspending two 5L beakers in a water bath at ≥95°C (Precision Scientific, Model 25, Chicago, IL). The treated whey was cooled to 20°C and transferred to the feed tank for microfiltration.

C. Processing.

1. Microfiltration Experiments

The experimental setup used in this research is shown in Figure 5. A variable speed peristaltic pump (MasterFlex®, Model # 7585-30, Cole-Parmer Instrument Co., Vernon Hills, IL) was used to pump whey and permeate

Table 11. Composition of Cottage Cheese Whey.

Component	Cottage Cheese Whey
Total solids (%)	6.32[a]
Moisture (%)	93.68
Protein (%) (N x 6.38)	0.75[b]
Fat (%)	0.02[b]
Ash (%)	0.60[a]
Calcium (mg/g)	1.36[b]

[a] average from six determinations
[b] average from 4 determinations

solutions through the microfilter and the ultrafilter, respectively. The different elements were conected by Tygon® B-44-4X tubing.

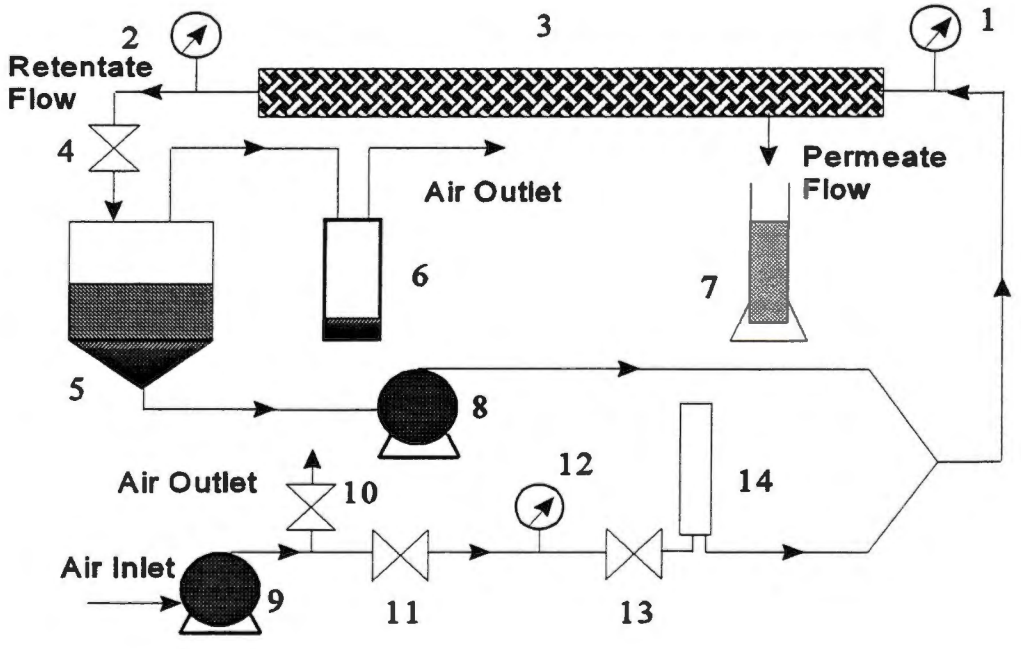

Figure 5. Experimental Setup for Microfiltration and Ultrafiltration.

1. inlet pressure gauge (P_{inlet}) 2. outlet pressure gauge (P_{outlet}) 3. cross-flow microfilter / ultrafilter 4. back pressure control valve 5. cheese whey reservoir 6. foam collector 7. permeate collector 8. peristaltic pump 9. air pump 10. air flow rate control valve. 11. check valve 12. pressure gauge (P_a) 13. air flow rate control valve 14. rotometer.

Microfiltration was carried out using a 0.1 μm pore size microfilter with 0.15 m^2 membrane area (A/G Technology Corporation, Needham, MA). This process was used to replace the clarification step needed before UF and also to remove residual whey lipids from raw whey, EDTA treated whey, and $CaCl_2$ treated whey. The membrane was throughly cleaned before each run. The permeate flux of pure water was used to check the effectiveness of cleaning. This is important to consider when comparing permeate flux after each whey pretreatment. Warm water (50°C) was used to wash the membrane for 20 minutes in a non-recycling mode. A 0.5N solution of NaOH at 50°C was recycled for 40 minutes, followed by flushing with warm water for another 40 minutes. This procedure was provided by the membrane manufacturer. Concentration during filtration was calculated as volume reduction (VR):

$$VR = \frac{\text{Volume Permeate}}{\text{Initial Feed Volume}} \times 100$$

To compare the effect of the liquid flow rate on flux, three flow rates were used (5, 10 and 20 L/min). Permeate flux was determined by the following formula after collecting the permeate in a graduated cylinder and timing with a stopwatch:

$$\text{Flux} = \frac{\text{Permeate Flow}}{\text{Membrane Area}}$$

All runs were made at 20°C and 10 psi average transmembrane pressure (TMP), defined as:

$$TMP = \frac{P_{inlet} + P_{outlet}}{2} - P_{permeate}$$

Since the permeate was always exposed to the atmosphere, the value of $P_{permeate}$ was assumed to be zero. An air pump (Gast® Model # DOA-P104-AA) was used to inject air bubbles into the liquid flow and achieve a two-phase cross flow during MF. Three air percentages (0,10 and 20%) were set by monitoring the air flow in the rotometer and the air pressure. Permeate fluxes were measured for the untreated whey at three flow rates (5, 10, 20 L/min). Permeate fluxes were also measured for the untreated and treated whey at a flow of 10 L/min, with and without application of air.

2. Ultrafiltration Experiments.

Microfiltration permeates (15 to 20 L) from raw, EDTA, and $CaCl_2$ treated whey were ultrafiltered in a 10,000 nominal MWCO hollow fiber polysulfone membrane with 0.28 m^2 of membrane area (A/G Technology Corporation, Needham, MA). Operating parameters were 20°C, 10 L/min flow rate and 10 psi TMP. The purpose was to compare permeate fluxes for the different microfiltrates, with and without air, as well as concentrate the whey proteins.

Concentration was calculated as the volume concentration ratio (VCR):

$$VCR = \frac{\text{Initial Feed Volume}}{\text{Retentate Volume}}$$

Permeate flux and TMP were determined and calculated as previously discussed. Air was introduced to the liquid flow at 0 and 20% to see the effect of two-phase cross flow on the UF permeate flux. Cleaning procedures between runs consisted of flushing with clean water for 20 minutes, recycling a 0.5N solution of NaOH for 20 minutes, flushing again with clean water for 15 minutes, circulating a solution of 100 ppm of NaOCl for 1 hour and finally rinsing with clean water for 20 minutes. This procedure was provided by the manufacturer. The goal was to recover the water permeate flux of 250 mL/min at 25°C and 10 psi TMP, reported by the manufacturer.

D. Chemical Analyses.

1. Total Solids.

Total Solids were determined using an atmospheric oven drying method (AOAC, 1980). Five grams of sample were dried in a vacuum oven (Baxter, Scientific Products, Model # 7595 -1) at 100°C and atmospheric pressure for 5 hours. After cooling the samples in a desiccator, they were re-weighed.

Moisture was calculated as the water loss during the drying period. Total solids were determined as:

$$\%TS = 100\% - \%Moisture$$

2. Ash.

The ash content of cottage cheese whey was determined by a modified AOAC (1980) method. Dried samples from the total solid determination were ashed in a furnace chamber (Thermolyne Corporation, Model # F-A1730, Dubuque, Iowa) at 625°C over 16 hours. Ash content was calculated as:

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

3. Calcium.

Calcium was determined by wet digestion, using a modified atomic absorbance spectroscopy method (Pollman, 1991). Whey samples were brought to room temperature, thoroughly mixed and pipetted into previously weighed beakers in 2 gram portions. 60 mL of HNO_3 were added and the samples were placed on a pre-heated hot plate, located inside a perchloric acid

approved fume hood. The samples were allowed to boil until the nitric acid was almost dry. Then, they were cooled, 7 mL of perchloric acid (70%) were added, and they were brought back to boil until there was 1 to 2 mL of perchloric acid. Samples were removed from the hot plate, cooled at room temperature and a series of dilutions were made, until the samples fit the concentration range used on the atomic absorption unit. A 0.1% lanthanum oxide solution (a phosphate inhibitor) was included in the last dilution, and the samples were analyzed on an Atomic Absorption and Atomic Emission Spectrophotometer (Allied Analytical Systems, Wathan, MA). Calcium absorption was determined at 422.7 nm.

4. Total Lipids.

A method modified from the work of Bligh and Dyer (1959) was used to determine total lipids. Prior to lipid extraction, whey samples were thawed in a water bath (25°C) and throughly mixed. A 20 mL sample was transferred into a 60 mL stainless steel container, and 30 mL of methanol were added. The mixture was homogenized at low speed for 1 min with a Virtis Model 23 homogenizer (The Virtis Company, Inc., Gardiner, NY). After this step, 20 mL of chloroform were added to the previous mixture, which was homogenized at the same speed for 1 min. After addition of 20 mL of an aqueous zinc acetate solution (0.115 g zinc acetate/5 mL) and homogenization for 10 seconds, the mixture was filtered through Whatman No 1 filter paper (Fisher, Pittsburg, PA)

using a 250 mL flask. The filter was removed and the flask was rinsed with 10 mL chloroform and placed back into the homogenizer for 1 min. The extract was filtered through Whatmam No 1 filter paper into the 250 mL flask. The homogenizer flask and filter were rinsed with 10 mL of chloroform, and the mixture was poured into a 100 mL graduated cylinder after rinsing the homogenizer flask with 2 mL of methanol. The cylinder was covered, kept in a cooler at 4 - 6°C, until the two phases were clearly separated (24 hr), and the volume of the lower layer (chloroform) was recorded. The cylinder contents were poured into a 250 mL separatory funnel and allowed to set in a cooler (4 - 6°C) for 2 hours. The bottom layer was drained into a 50 mL Erlenmeyer flask, and a 10 mL aliquot of the extract was pipetted to a dry and tared beaker, and allowed to evaporate to dryness overnight under a hood. The next day, the beaker was dried at 400°F in an oven for 30 min (Will Corporation, Rochester, NY), cooled in a dessicator and re-weighed to determine the total lipid content by the formula:

$$\text{Total Lipid (\%)} = \frac{(\text{lipid wt.})}{(\text{vol. aliquot})} \frac{(\text{vol. } CHCl_3)}{(\text{sample wt.})} \times 100$$

5. Total Protein.

A modified AOAC (1984) Kjeldahl method was used to determine the total

protein content of cottage cheese whey samples. Liquid 10 mL aliquots were pipetted into a Kjeltec digestion tube, followed by addition of 2 Keltabs ($CuSO_4$),15 mL of H_2SO_4 and mixing. The sample was placed in a Digester DS 6/20 (Tecator Analytical Company, Höganäs, Sweden) and allowed to digest under a hood by slowly increasing the temperature to 420°C over a period of 6 hours to avoid splattering. After the sample was cooled it was placed in a Kjeltec 1026 Distilling Unit (Tecator Analytical Company, Höganäs, Sweden), and 25 mL boric acid solution were added to a receiver flask. The receiver flask was placed on the platform of the distilling unit and the distillation procedure ran automatically. Titration of the receiver flask solution was made to neutral gray endpoint with 0.1N HCl, and the volume of acid was recorded. The % nitrogen and protein were determined using the following formulas:

$$\% \ N = \frac{(0.1401) \times (\text{mL titrant sample} - \text{mL titrant blank})}{\text{grams of sample}}$$

$$\% \ \text{Protein} = \% \ N \times 6.38$$

6. Protein Permeation.

Protein permeation was calculated as the ratio between protein concentration in the permeate (Pp) and protein concentration in the feed (Po), where a value of 1 means that no protein has been rejected by the membrane or

0% rejection:

$$\text{Protein Permeation} = \frac{P_p}{P_o}$$

7. SDS-PAGE.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to degrade and fractionate whey proteins from raw, pretreated whey, MF permeates and UF retentates processed with and without the application of air. An equal volume (100 µL) of whey sample and 2X sample buffer (12.5% 0.5 M Tris pH 6.8, 4% SDS, 20% glycerol, 0.02% bromophenol blue solution, 10% mercaptoethanol and 12.5% water) was mixed, heated for 10 min at 95°C and cooled to 20 - 25°C. Twenty µL of sample, the blank and a wide range molecular weight (6,500 to 205,000 D) Sigma Marker Standard (Sigma Chemicals, St. Louis, MO) were loaded into a 15% polyacrylamide gel. The procedure was completed on a Hoefer SE 600 Series vertical slab gel electrophoresis unit (Hoefer Scientific Instruments, Model PR 70/75, San Francisco, CA) connected to an EC 3000 P Series 90 Programable power supply unit (EC Apparatus Corporation, St. Petersburg, FL). Gels were released from the plates and stained overnight in a staining solution which contained 0.025% Coomassie Brilliant Blue R250, 50% methanol and 9.2% acetic acid. The next

day, gels were destained for a period of 24 to 48 hr in a destaining solution containing 10% methanol and 7.5% acetic acid.

E. Statistical Analyses.

A Randomized Block Design (RBD) blocked on whey with a fractional factorial treatment arrangement (from 3 types of pretreatment, 3 levels of flow rate and 3 levels of air) was used to find the best combination of flow rate, air percentage, and pretreatment method to achieve the highest permeate flux after 1 hour of operation in the microfilter. This statistical design did not include all possible treatment combinations, but only those necessary to estimate main effects. Data were analyzed with weighted analysis of variance using Proc Mixed (SAS Inc., Cary, NC). The 'pdmix612.sas' algorithm from the SAS software package (Saxton, 1998) was used to generate the letter group separation of the significant differences among treatments. Orthogonal Polynomial Contrasts (SAS Inc., Cary, NC) were used to test specific hypotheses concerning the 'whey main effect', the 'air main effect', the 'flow rate main effect', and the interaction 'interaction effect of whey*air' in the microfiltration tests.

A Randomized Block (RBD) with a factorial treatment arrangement (3 types of whey pretreatment by 2 levels of air) was used as statistical design to find significant differences in protein permeation among the treatments, after 1

hour of operation in the microfilter. Data were analyzed with a procedure called Proc Mixed (SAS Inc., Cary, NC). The 'pdmix612.sas' algorithm was used to generate the letter group separation of the significant differences among the treatments and interactions.

A Complete Randomized Design (CRD) with a factorial treatment arrangement (3 types of whey pretreatment by 2 levels of air) was used as statistical design to detect significant differences in the permeate flux during the ultrafiltration experiments. Data were analyzed by Proc Mixed, and the letter groups of the significant differences among the treatments and for interactions were generated by 'pdmix612.sas'.

CHAPTER IV

RESULTS AND DISCUSSION

A. Microfiltration Experiments.

1. Microfiltration Flux Rates.

The purpose of this research was to determine the effects of whey pretreatment, flow rate, and air injection on the permeate flux of cottage cheese whey solutions. The data obtained from the statistical analysis considered values of permeate flux during the first hour of microfiltration (Appendix A-1).

The results obtained showed that changing the flow rate from 5 to 10 L/min for untreated whey, without introducing air in the system, led to a 52% increase in the mean flux. A change in flow rate from 10 L/min to 20 L/min resulted in a 32% increase. When 10% air was introduced to the microfiltration unit, operating at 10 L/min, mean flux was increased 49%. The application of 20% air for the same flow rate resulted in a 52% flux increase, showing similar results to those obtained by increasing the flow rate from 5 to 10 L/min. Considering only the effect of whey pretreatment at 10 L/min flow rate and 0% air, mean permeate flux decreased in the following order: $CaCl_2$ treated whey > EDTA treated whey > Raw whey (Figure 6). In addition, introducing 20% air when processing these whey solutions revealed that a higher mean permeate

flux increase was obtained for raw whey than for EDTA whey, with 52% and 49% respectively. $CaCl_2$ whey mean permeate flux increased only 28% with addition of 20% air. This indicates that the application of air was more effective when fouling due to concentration polarisation was worse, which was the case for untreated whey.

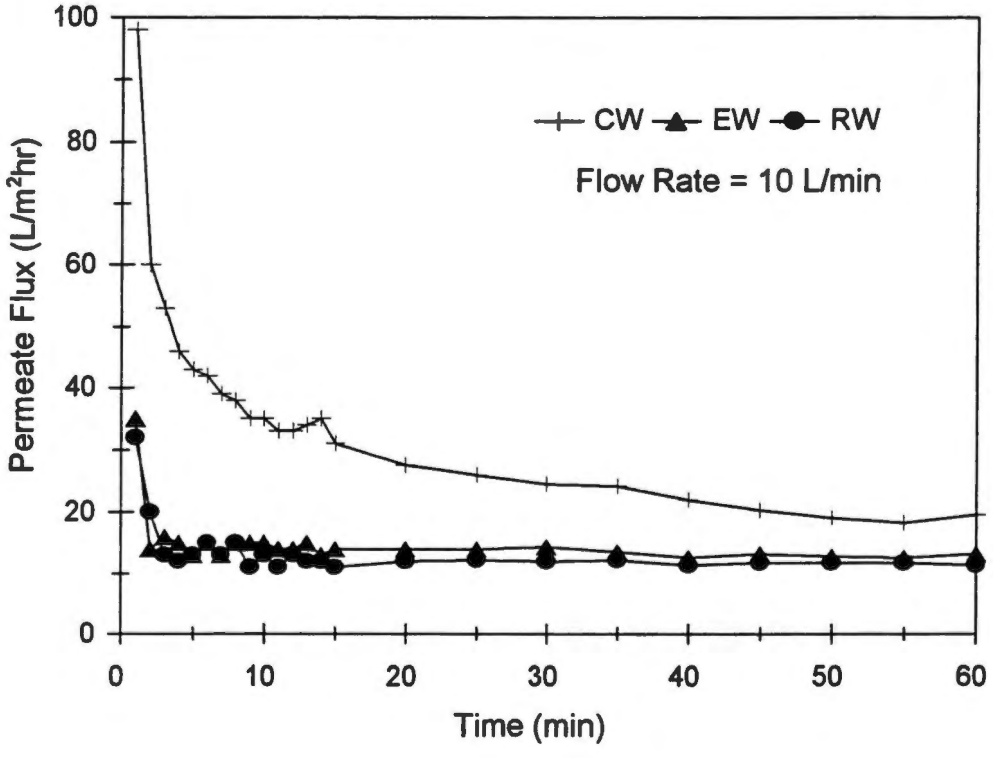

Figure 6. Effect of Pretreatment on Permeate Flux during Microfiltration. (CW = calcium chloride treated whey; EW = EDTA treated whey and RW = untreated whey).

Improvements in permeate flux during cross flow microfiltration with application of 20% air for untreated and treated whey solutions can be seen in Figures 7, 8 and 9.

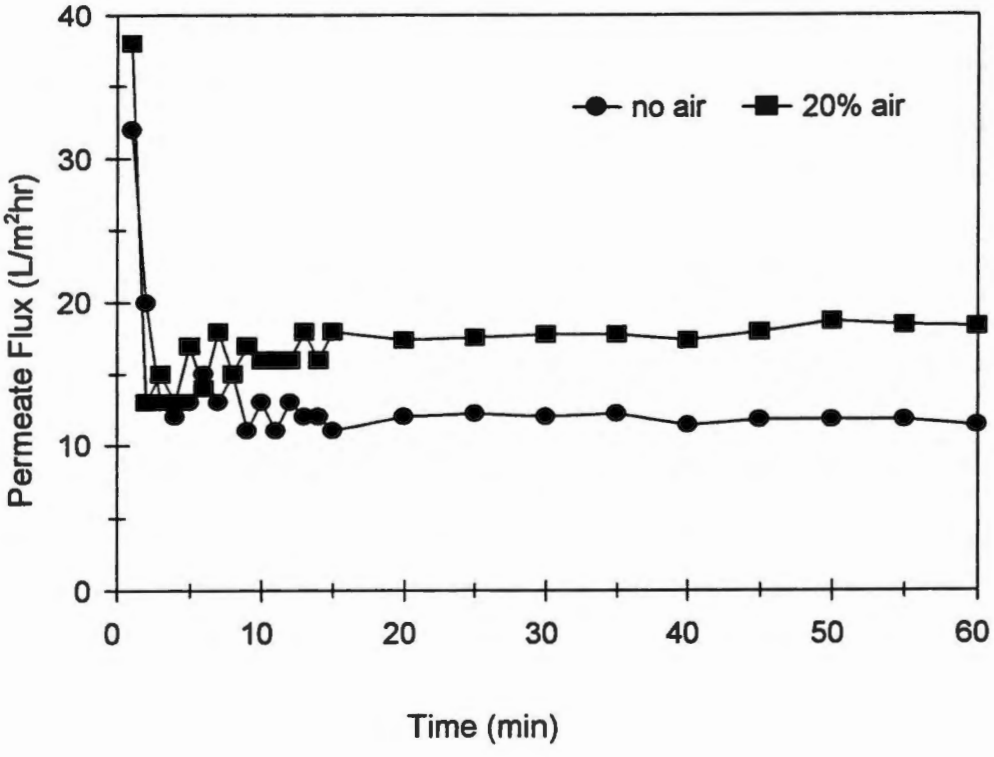

Figure 7. Effect of Air on MF Permeate Flux for Raw Cottage Cheese Whey (Flow rate = 10 L/min).

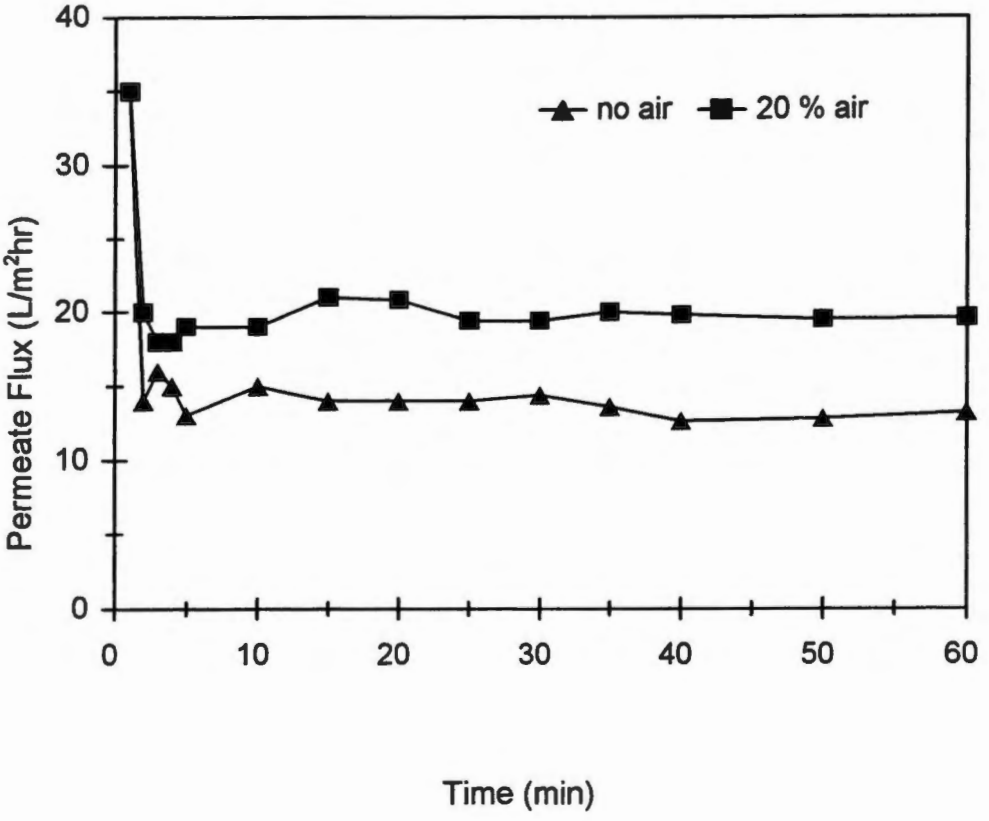

Figure 8. Effect of Air on MF Permeate Flux for EDTA treated Whey. (Flow rate = 10 L/min).

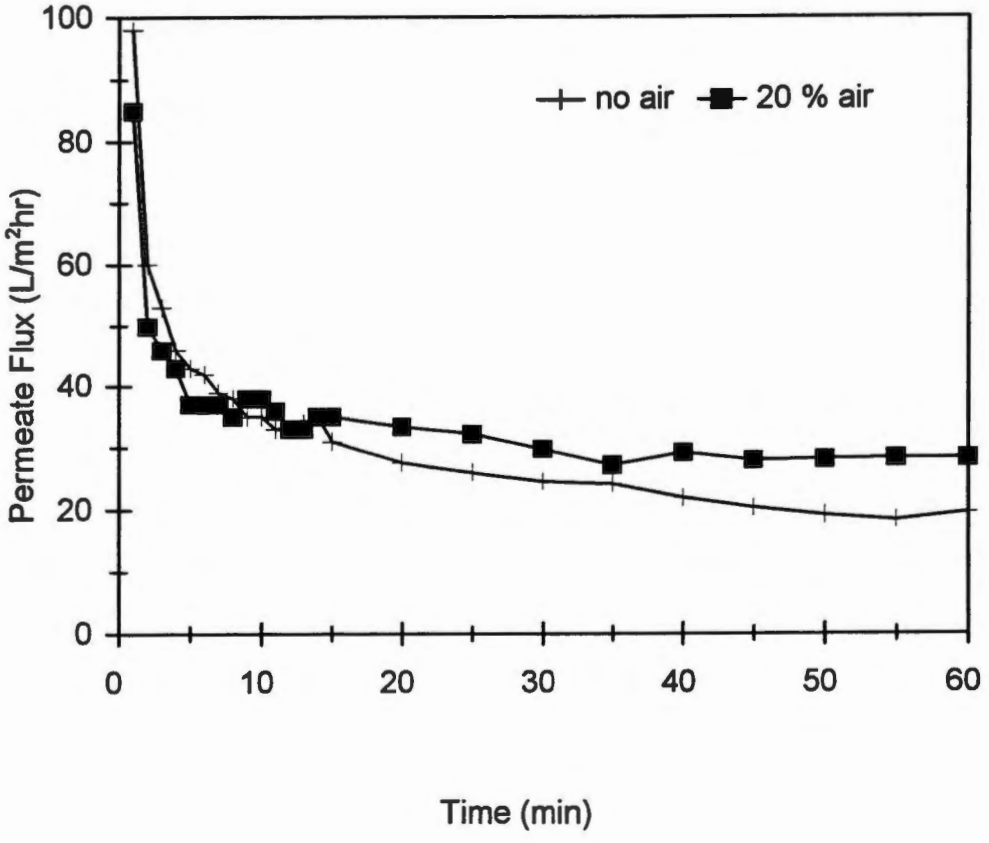

Figure 9. Effect of Air on MF Permeate Flux for $CaCl_2$ treated Whey. (Flow rate = 10 L/min).

There was a significant difference ($p < 0.05$) among LSmeans of flux for RW at 10 L/min flow rate with no air injection, and with the application of either 10% or 20% air. Significant differences ($p < 0.05$) were also found between LSmeans for EW flux (no air) and 20% air, nevertheless, this difference was not present for CW. In addition, no significant difference was found between the injection of 10% and 20% air. The highest mean flux obtained was 29.96 L/m^2hr for CW with 20% air (Appendix B-1). As expected, the lowest mean flux was obtained for RW at 5 L/min without injection of air. Increasing the flow rate from 10 L/min to 20 L/min for RW without injection of air did not show significant differences ($p < 0.05$) (Table12).

Table 12. Effect of Pretreatment, Flow Rate and Air Percentage on Permeate Flux (L/m^2hr) of Cottage Cheese Whey during Microfiltration.

Flow Rate (L/min)	Whey Pretreatment						
	Raw Whey			EDTA Whey		$CaCl_2$ Whey	
	Air (%)			Air (%)		Air (%)	
	0	10	20	0	20	0	20
5	7.72^{d}						
10	11.76^{cd}	17.50^{ab}	17.83^{ab}	13.44^{c}	19.96^{a}	23.3^{abc}	29.96^{a}
20	15.58^{bc}						

* LSmeans with different letters differ ($p < 0.05$)

Orthogonal contrasts were also used to see the effect of parameters such as flow rate, air, and type of whey in the experiment. These contrast statements revealed that the "whey main effect" and "air main effect" were significant ($p<0.05$) (Appendix A-2).

The effect of two-phase cross flow microfiltration seems to depend on the level of fouling during processing of cottage cheese whey. Unclarified or raw whey, which contains casein fines, whey proteins, lipids and minerals among other severe foulants, showed the highest increase in permeate flux when air was applied to the microfilter. This suggests that air is more effective when fouling is the result of severe concentration polarisation. In addition, no significant differences ($p<0.05$) in mean flux were found between 10% and 20% air, suggesting that only a low percentage is needed when the goal is to increase permeate flux of whey solutions. In other words, a small amount of air bubbles provide the necessary shear force to reduce the fouling problem. Cui and Wright (1996), had previously shown that this enhancement effect was more profound when fouling was severe and less significant with turbulent liquid flow, even though they did not support their results with statistical analysis. A flow rate of 10L/min falls into the laminar region for the microfilter in our experiments.

2. Volume Reduction.

The rate of concentration during the microfiltration experiments was

higher for the two-phase cross flow method of operation when using the same initial feed volume and operating parameters. This had the advantage of shortening the operating time. Figure 10 shows the times needed to achieve a 60 % VR of different whey solutions.

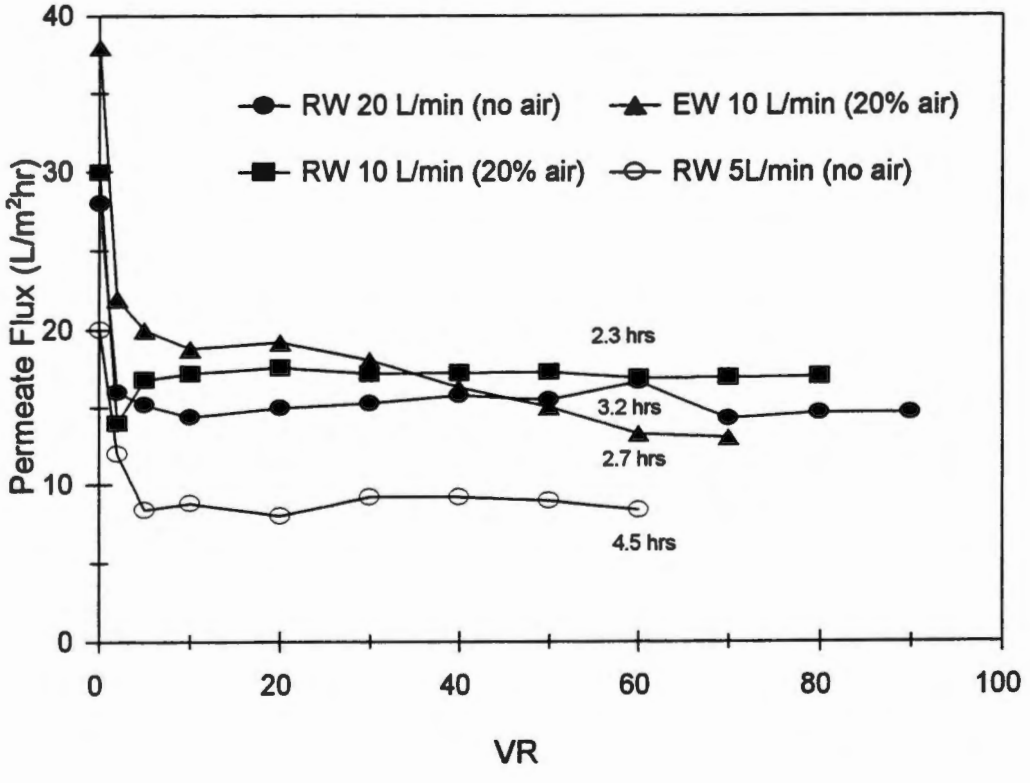

Figure 10. Operating Times Needed for a VR of 60%.

Although concentration during the microfiltration experiments was not an objective of this study, the considerable reduction in process time observed for most of the runs in which air was applied to the unit is a great advantage. As stated before, increasing flow rate can also increase permeate flux and decrease time requirements, but the two-phase method is more efficient in terms of energy input.

3. Calcium Permeation.

Initial levels of calcium for untreated and treated whey are shown in Table 13. Pretreating whey with $CaCl_2$ increased the calcium content by 24 % and left a clear supernatant, resembling microfiltration permeates (Figures 11 and 12).

The application of 20% air not only increased permeate flux during microfiltration, but also improved calcium permeation. Atomic absorption analysis of calcium revealed that this mineral, considered a severe foulant in the whey, was better permeated when microfiltration was carried out in the two-phase mode for any of the pretreatments. Calcium analysis of microfiltration permeates taken after 1 hour of operation is shown in Figure 13.

According to Rao et al. (1994a), most of the calcium in whey is present in a soluble form, nevertheless, it did not permeate freely during their microfiltration experiments. Our data also reveals a decrease in Ca content in all

Table 13. Atomic Absorption Analysis of Calcium in Whey.

Pretreatment Modificatiion	Calcium content [a] (mg/g)
Raw Whey	1.36
EDTA Whey	1.31
$CaCl_2$ Whey	1.68
$CaCl_2$ Supernatant	0.18

[a] average from two determinations

microfiltration permeates, confirming that calcium plays a significant role in the fouling of these membranes. Rao et al. (1994b) also observed a continued flux drop in time during UF of some dairy products. They stated that the precipitation of soluble calcium phosphate as well as adsorption of proteins were responsible for the flux drop in sweet whey solutions.

Calcium rejection values obtained after one hour of process are shown in Table 14. Notice that air always improved calcium permeation, and this is reflected as lower rejection values for both, treated and untreated whey. The high rejection values obtained for calcium chloride treated whey are a consequence of the pretreatment modification applied, in which Ca precipitates with lipoproteins as complex phosphate salts, which are separated from the whey through microfiltration. In this case, the initial calcium content was reduced by nearly 80% in the first hour of microfiltration, in spite of the added calcium.

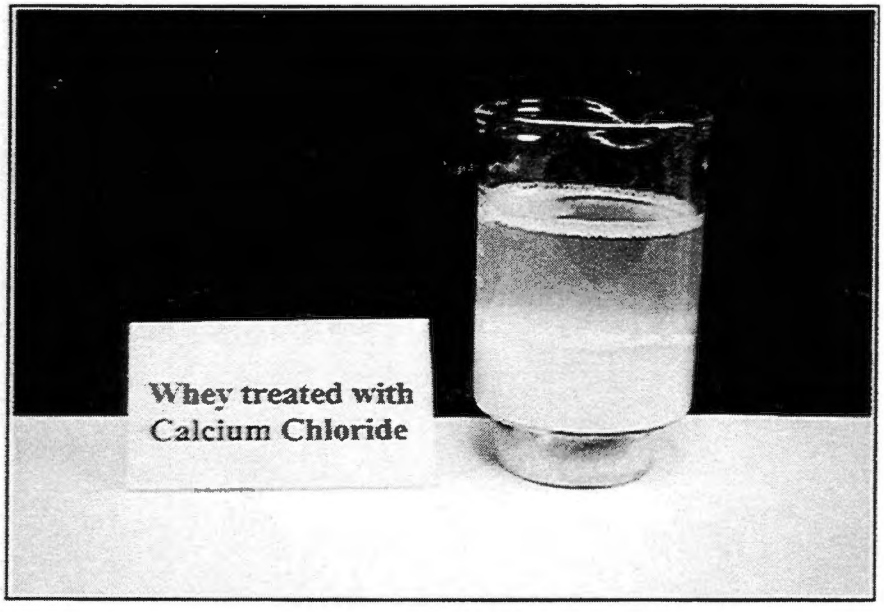

Figure 11. Precipitation observed after Whey Pretreatment with Calcium Chloride.

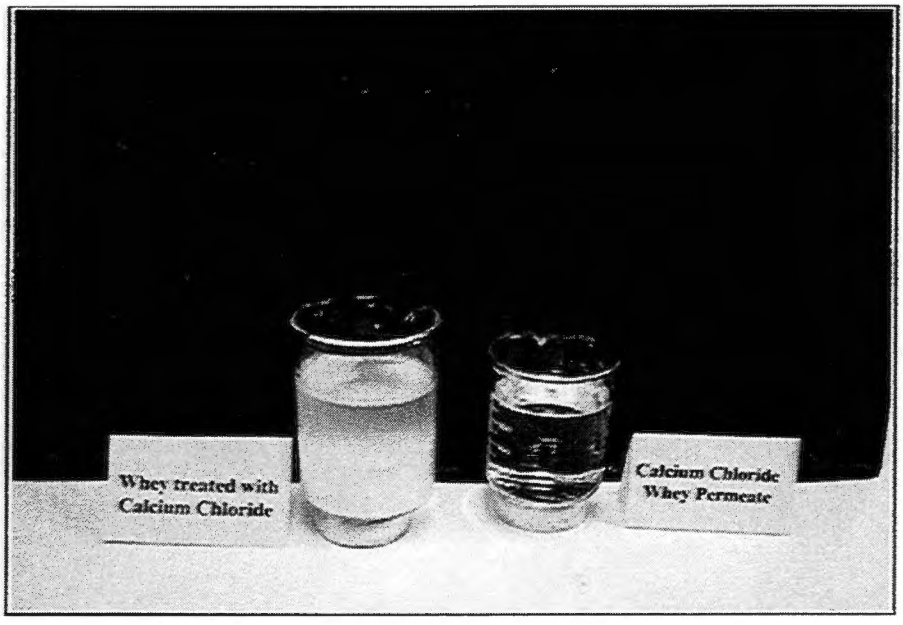

Figure 12. Comparison between Calcium Chloride Pretreated Whey Supernatant and the Microfiltration Permeate obtained after Processing.

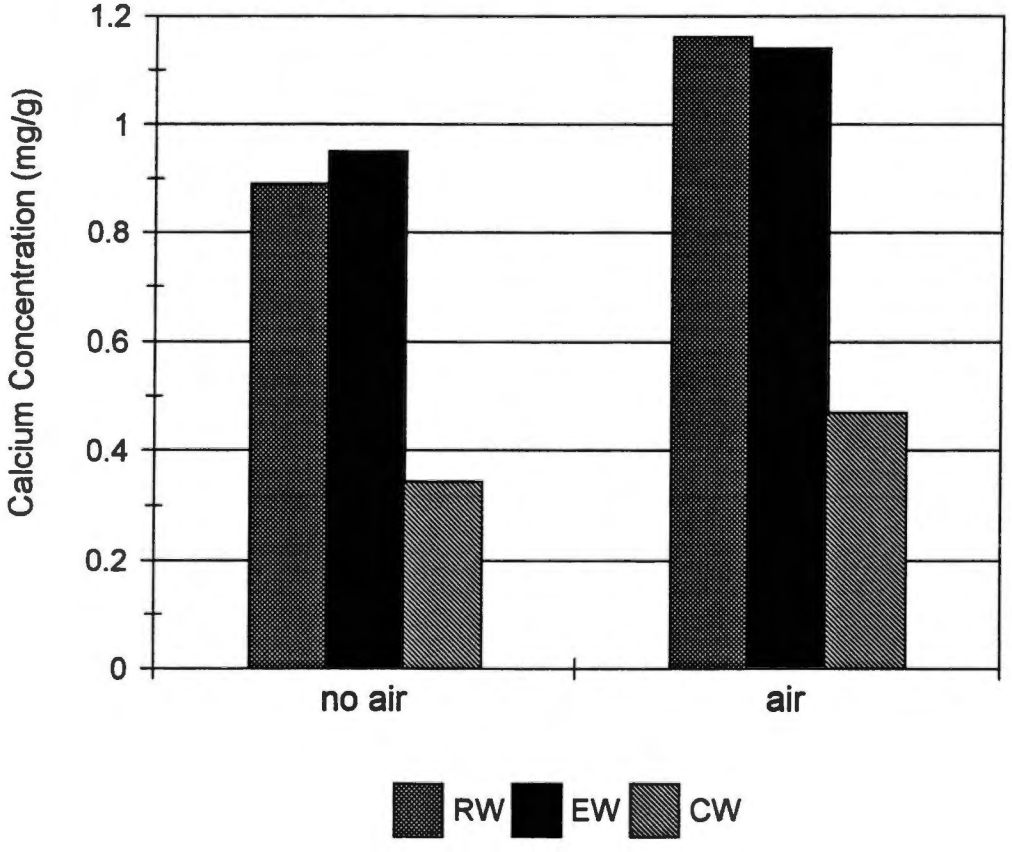

Figure 13. Calcium Concentration in MF Permeates taken after one Hour of Operation.

Table 14. Calcium Rejection Values for MF Permeates obtained with and without the application of air.

Whey	Rejection Values*	
	no air	air
RW	0.348	0.147
EW	0.274	0.129
CW	0.796	0.720

* values calculated for permeates taken after one hour of microfiltration.

By applying air during whey microfiltration, calcium permeation increased from 65.2% to 85.3% for raw whey, from 72.5% to 87% for EDTA treated whey and from 20.4% to 27.9% for $CaCl_2$ treated whey. The fact that more calcium was permeated in the two-phase cross-flow method of microfiltration suggests that some of the rejected soluble calcium could also be involved in the fouling process.

Addition of EDTA, with pH adjustment was not as effective as expected. Many authors have suggested that calcium, present in acid whey mainly in the ionic state, may be sequestrated by this chelating agent to improve flux rates. Nevertheless, the use of either an excess or not enough EDTA can also be detrimental in some cases. In our experiment probably not all the soluble calcium was sequestrated by addition of EDTA, because of the variability of

initial calcium content in the whey buckets. This could explain why so much calcium was permeated during the first hour of microfiltration, as well as the relatively low flux enhancements obtained with addition of EDTA. Patocka and Jelen (1987), obtained the highest increase in flux by adding EDTA in the ratio 1 meq/1meq Ca during ultrafiltration of cottage cheese whey. According to the authors, the amount of chelating agent used should be equivalent to the amount of free calcium in the whey. In addition, the specific equilibrium between colloidal and ionic calcium in the whey also has an effect on the permeation rates obtained by using chelating agents such as EDTA, and the final pH of the solution will determine that specific equilibrium.

4. Protein Permeation.

Statistical analysis revealed that there were significant differences ($p<0.05$) in protein permeation among whey treatments (Appendix A-3 and B-2). The air effect and the interaction between whey and air were also significant ($p<0.05$). Results are shown in tables 15, 16 and 17.

Table 15. Effect of Whey Pretreatment on the Protein Permeation of Whey Proteins.

Whey Main Effect		
RW	EW	CW
0.4960^{c}	0.5404^{b}	0.6443^{a}

*LSmeans with different letters differ ($p<0.05$)

Table 16. Effect of Air Percentage on the Protein Permeation of Whey Proteins.

Air Main Effect	
0%	20%
0.4802^{b}	0.6403^{a}

*LSmeans with different letters differ ($p<0.05$)

Table 17. Interaction between Whey and Air Main Effect on the Protein Permeation of Whey Proteins.

	Whey Pretreatment		
Air %	RW	EW	CW
0	0.4387^{f}	0.4780^{e}	0.5238^{d}
20	0.5533^{c}	0.6029^{b}	0.7648^{a}

*LSmeans in the same row or column with different letters differ ($p<0.05$)

Even though individual protein permeation was not determined, the above values for total protein permeation support the theory that fouling during microfiltration of cottage cheese whey is probably due to irreversible concentration polarization, in which proteins form sheets that affect the microfiltration rate and their permeation. Both, α-lactalbumin and β-lactoglobulin are much smaller than the 0.1μm pore size of the MF membrane, nevertheless, these proteins did not permeate freely. By applying either chemical or physical pretreatment, or the combination of both, the values obtained always improved.

In our experiment, many factors could have contributed to the relatively low protein permeation values. The fouling phenomena was greatest during MF of untreated (raw) cottage cheese whey, resulting in the lowest protein permeation value. In addition, it is known that flux rates can be improved not only by pretreatment modifications, but by processing at higher temperatures, allowing higher permeation rates for whey proteins (Knopp, 1992). Our experiments were carried out at 20°C, for all runs, in an attempt to eliminate temperature from affecting statistical differences and also to reduce energy requirements during the filtration process. Therefore, lower values than those reported in the literature were expected.

The effect of pH and ionic strength on protein permeation is evident. Protein permeation was improved when processing at higher pH. Higher values were obtained for $CaCl_2$ whey, where the pretreatment modification resulted in a final pH of 7.1 in the solution to be processed.

According to Marshall et al (1993), maximum deposition of proteins occurs around the isoelectric point. Most of the whey proteins have their isoelectric point near 5.0, where they are least soluble and more susceptible to aggregation. Since both, the membrane and the state of the protein affect the degree of protein deposition, an increase in the pH away from the IEP (isoelectric point) probably resulted in less deposition. At higher pH, the major whey proteins have a negative net charge and enlarge due to electrostatic repulsion.

The literature also reveals that particularly BSA and β-lactoglobulin are sheet forming proteins during MF and UF, thus drastically hindering permeation. Other studies have shown that BSA accumulates not only at the membrane surface but within the pores of 10,000 D polysulphone membranes. Dauffin et al (1991) reported retention of 100% of BSA and Ig, 50% of lactoferrin and lactoperoxidase and approximately 30% of β-lactoglobulin and α-lactalbumin during MF of untreated whey obtained from rennet casein production when using an inorganic membrane. BSA is also known to interact with other whey proteins, such as lactoferrin, lysozyme or α-lactalbumin, forming complexes which could be larger than the MWCO of the membrane under study. On the other hand, β-lactoglobulin has a tendency to polymerize below pH 8.0. All these factors could explain the low permeation rates during microfiltration.

Lee and Merson (1976a) previously stated that permeation rate could be improved if these proteins were maintained in a disperse state without allowing

them to deposit on the membrane.

In our experiments, application of 20% air had a significant effect ($p<0.05$) on the total protein permeation, as well as the interaction between whey and air, which gave a highest value of 0.7648 for calcium chloride treated whey and air. This particular pretreatment removed lipoproteins during microfiltration, minimizing membrane fouling. Researchers had observed that the presence of large molecules in a solution can increase the retention of smaller molecules. "Large molecules traveling through the narrow, tortuous confines of the porous membrane matrix are slowed down by friction with the pore wall and hinder the transport of smaller molecules." (Marshall et al., 1993). Removing lipoproteins from the whey solution resulted in an increase of solute and solvent permeating the membrane.

Previous studies done by Bellara et al (1996) showed that air sparging disrupts the concentration polarisation boundary layer during UF, enhancing permeate flux but reducing the sieving coefficient of single protein (BSA) solutions at concentrations of 2 g/l. Our results indicate that despite the low permeation rates during the MF of cottage cheese whey, application of air enhanced permeate flux by decreasing membrane fouling, leading to significantly higher protein permeation values.

5. Individual Proteins by SDS-PAGE.

The goal of this analysis was to monitor protein changes during processing of cottage cheese as a function of the different pretreatment modifications and under the influence of air. Individual protein bands were identified from MF/UF permeates and retentates of treated and untreated whey taken after one hour of filtration (Figures 14 and 15).

A 12% polyacrylamide gel, showed weaker bands for all microfiltration permeates when compared to the original whey, in which the bands of the major whey proteins were easily identified. In addition, no higher molecular weight proteins, such as BSA and Ig were present after the microfiltration process. This confirms the results obtained by Dauffin et al. (1991), who observed a 100% retention of BSA during their microfiltration process. The low protein permeation rates obtained before are also supported by this fact. In addition, there are no bands present for UF permeates, because the proteins are retained and concentrated in the retentate during this process.

The same protein bands are present for the cases in which air was applied to the membranes. It appears that a two-phase cross-flow method of operation does not have any effect on the whey proteins molecular weight, but a positive effect in α-lactalbumin and β-lactoglobulin permeation rates.

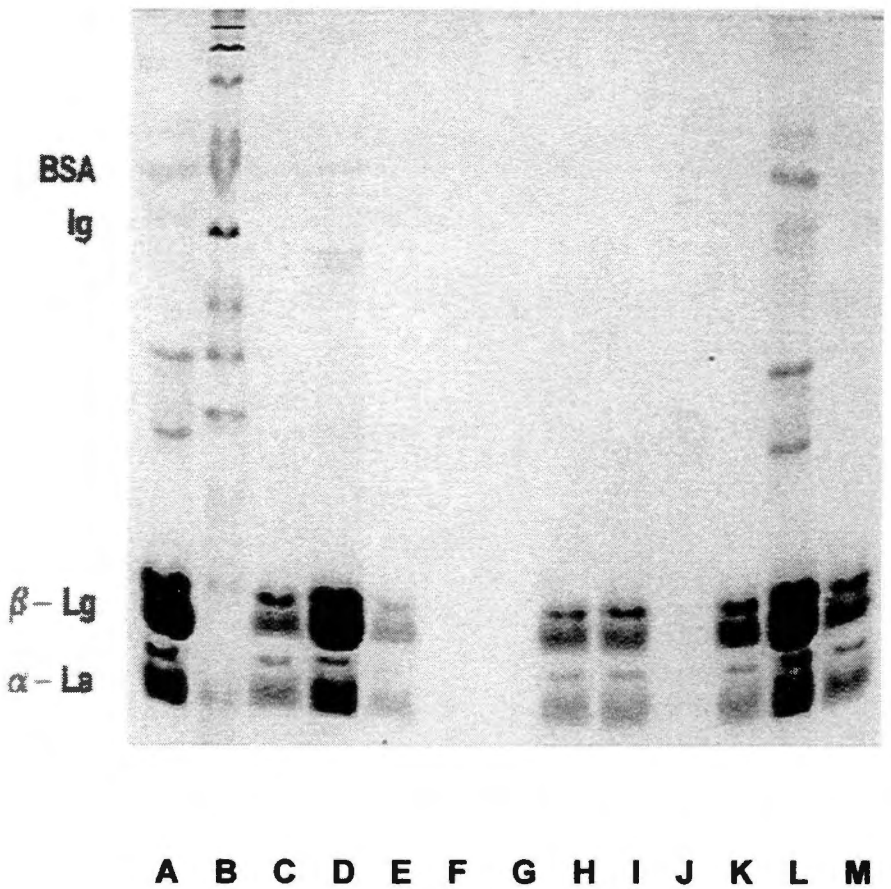

Figure 14. SDS-PAGE of MF/UF Whey Solutions

(A) RW; **(B)** Standards; **(C)** MF permeate from RW; **(D)** Supernatant from CW pretreatment; **(E)** MF permeate from CW; **(F)** UF permeate from CW (air); **(G)** UF permeate from CW; **(H)** MF permeate from EW; **(I)** MF permeate from EW (air); **(J)** UF permeate from RW; **(K)** UF retentate from RW; **(L)** RW; **(M)** MF permeate from RW.

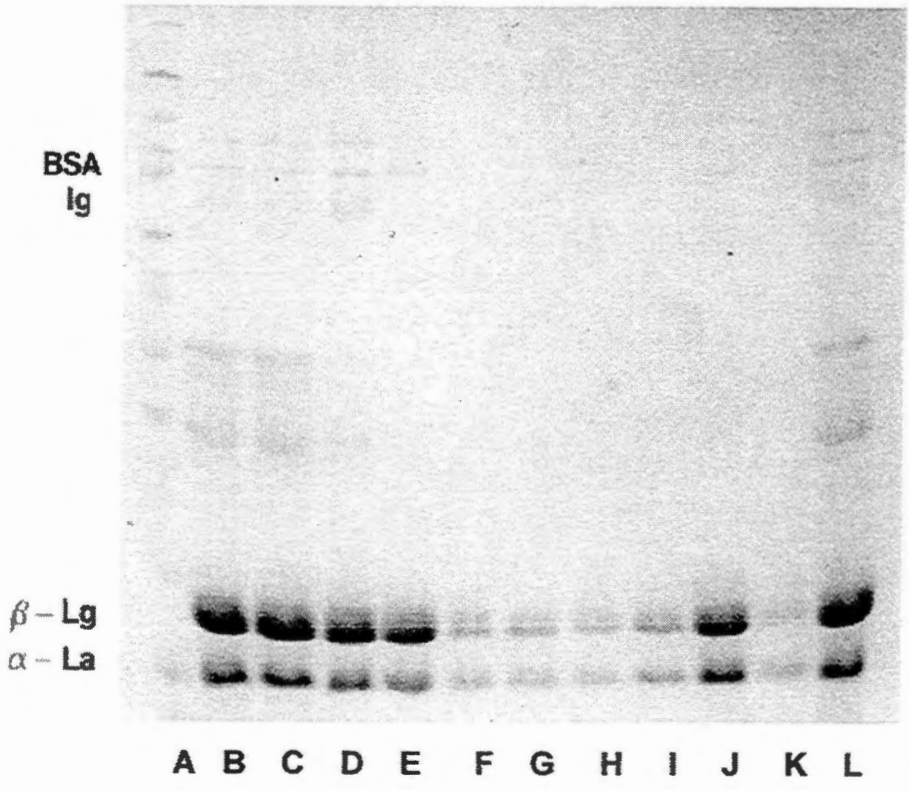

Figure 15. SDS-PAGE of MF Whey Solutions

(A) Standard; **(B)** RW; **(C)** EW; **(D)** CW; **(E)** Supernatant from CW **(F)** MF permeate from RW; **(G)** MF permeate from RW (air); **(H)** MF permeate EW; **(I)** MF permeate EW (air); **(J)** MF permeate from CW (air); **(K)** MF permeate from CW; **(L)** RW.

A 15% acrylamyde gel shows no difference among the protein bands obtained from any of the whey pretreatments. However, a difference in the intensity of the bands can be seen in the MF permeates for EW and CW (with and without air, respectively). This is consistent with the higher protein permeation results obtained for the two-phase cross-flow mode of operation.

6. Lipid Analysis.

The initial concentration of total cottage cheese lipids was around 0.02%. This concentration did not change significantly with the different pretreatment modifications, but was higher as the time of operation increased, as a result of a combined effect of clarification and concentration (Table 18).

As a consequence, the permeates obtained from the microfiltration process had very low lipid contents (less than 0.01%). According to the literature, lipids have a detrimental effect during MF/UF processes, causing the membranes to foul. Their removal has also been a focus of researchers due to a goal to obtain commercial WPC with residual lipids of less than 1%. In our research, the microfiltration process was successful in clarifying the whey and removing residual whey lipids (RWL) for all the pretreatment modifications. However, a lipid class analysis of these whey permeates would be necessary to compare residual lipid composition among pretreatment modifications. In particular, to monitor the complete removal of small milkfat globules and

Table 18. Change in Lipid Concentration of MF Whey Retentates through Time.

Process Time (hours)	Lipid Concentration [a] (%)		
	RW	EW	CW
0	0.025	0.027	0.028
1	0.028	0.029	0.029
5	0.051	0.057	ND
10	0.161	ND	ND

[a] average from two determinations.
ND = not determined

phospholipids (PLPC), which impair the UF process and limit WPC's quality and use. According to Vaghela and Kilara (1996), although residual lipids are very important in determining the use of WPC as a food ingredient, very little information is available from the literature. In addition, it is well known that MF removes RWL from cheese whey, but this has led to an incomplete recovery of the proteins in most cases. Further research is needed in the area of functional properties of defatted WPC's obtained by direct microfiltration.

B. Ultrafiltration Experiments.

1. Ultrafiltration Flux Rates.

The purpose of this research was to compare UF permeate fluxes among different feed solutions, with and without the application of air in the ultrafilter. The feed solutions consisted of the MF permeates obtained using the pretreatment modifications of the previous experiments. UF permeate flux rates were analyzed as a Complete Randomized Design with a factorial treatment arrangement (Appendix A-4 and B-3). Data obtained from the statistical analysis considered only values of permeate flux during the first hour of ultrafiltration for the two-phase cross-flow method of operation, due to problems caused by foaming of proteins. Differences in permeate flux resulting from processing the three types of MF permeates can be seen in Figure 16.

MF permeates from CW showed the highest permeate flux during the ultrafiltration experiments, followed by EW and RW. Treating whey with calcium chloride before microfiltration and using this permeate as a feed for ultrafiltration of whey proteins is beneficial in relation to permeate flux, as other authors have previously stated. No important differences in UF permeate flux were evident from using EDTA or untreated whey before microfiltration. Nevertheless, our results show that great flux enhancements can be obtained by applying air during the UF of these MF permeates, which can be seen in Figures 17, 18 and 19.

Significant differences ($p<0.05$) were obtained among LSmeans of UF permeate flux for the whey main effect, air main effect and the interaction between whey*air. These results show that changing from 0% to 20% air has

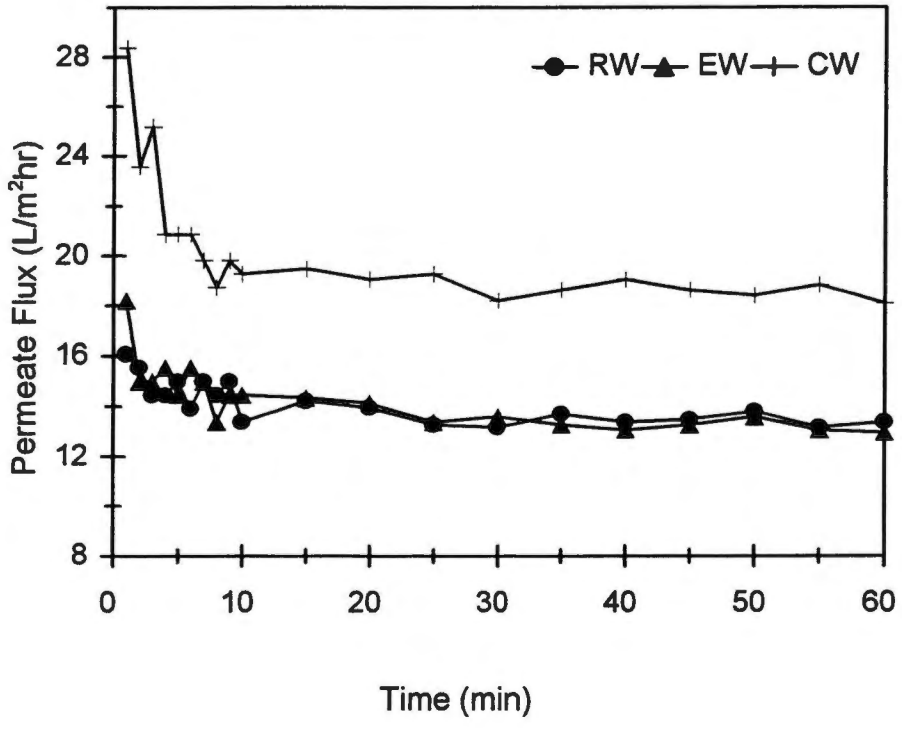

Figure 16. Effect of Pretreatments on UF Flux of Microfiltration Permeates
(RW = untreated whey; EW= EDTA treated whey; CW= Calcium Chloride treated whey).

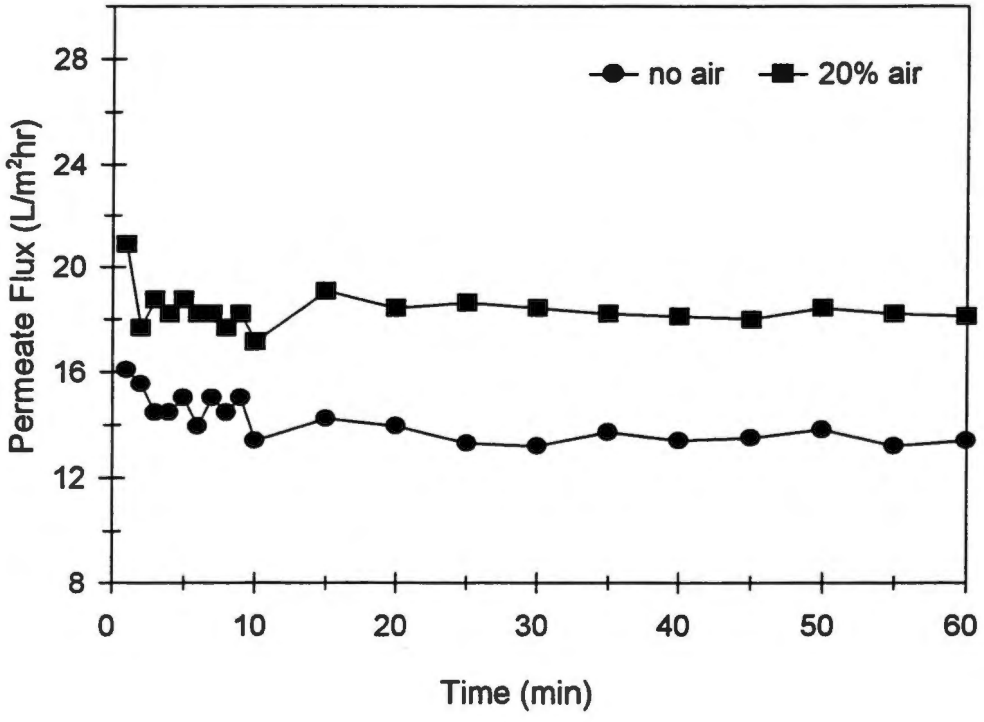

Figure 17. Effect of Air on UF of RW Microfiltration Permeate.

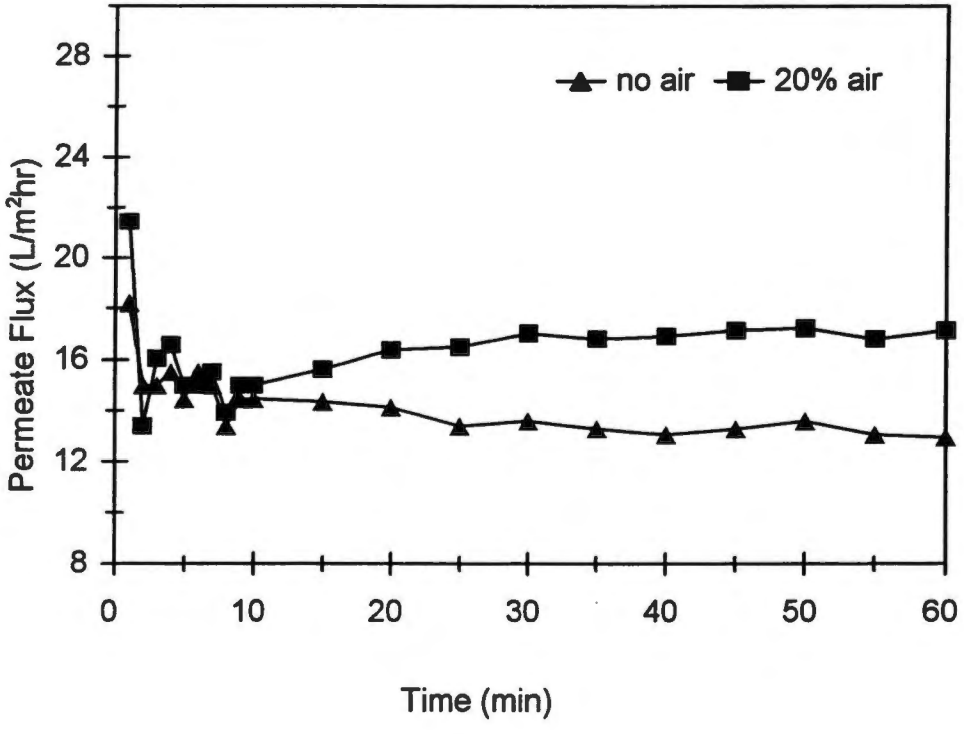

Figure 18. Effect of Air on UF of EW Microfiltration Permeate.

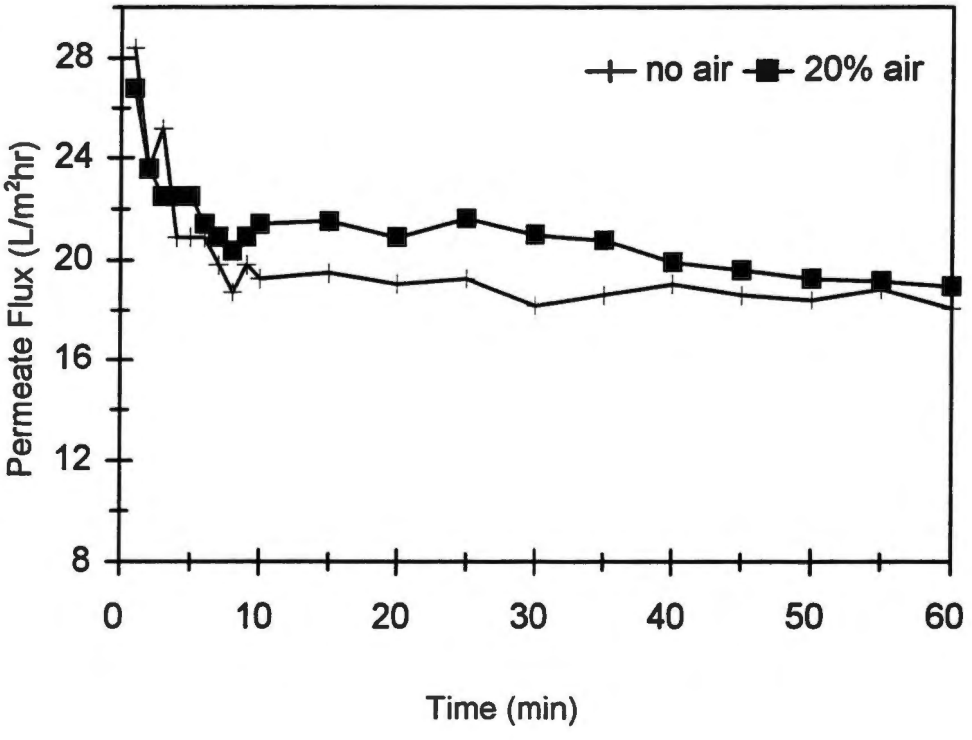

Figure 19. Effect of Air on UF of CW Microfiltration Permeate.

a significant effect on the LSmeans of permeate flux for each of the feeds used in the experiment (Table 19, 20, 21). In addition, applying 20% air to the microfiltration permeate of RW is as effective as processing microfiltration permeate of CW without air. Therefore, flux enhancement could result in reduced processing time for the two-phase cross-flow mode of operation. These results apply to the conditions of this experiment, in which the operating parameters were kept the same for all runs and replicates.

As expected, flux enhancement was more significant when processing microfiltration permeate from raw whey (34.3% compared to 10% for MF permeate from CW whey). As mentioned before, air seems to disrupt the concentration polarization layer formed by protein solutions, which seems to be more pronounced when processing raw whey. There was not a significant difference between the UF permeate fluxes obtained from RW and EW microfiltration permeates when air was not applied. Therefore, the EDTA pretreatment modification did not have an enhancing effect during ultrafiltration. In addition, mean values obtained from RW microfiltration permeates were higher, both with and without air.

As stated before, no significant difference was observed between the permeate flux obtained for RW microfiltration permeate (20% air) and CW microfiltration permeate (no air), which were 18.24 L/m^2hr and 18.69 L/m^2hr, respectively. During microfiltration experiments, these two permeates reflected similar values for protein permeation, with RW MF permeates (20% air)

significantly higher than CW MF permeates (no air). These results are important and suggest that the calcium chloride pretreatment modification could be replaced in the future by the sole application of air during processing of cottage cheese whey. One important factor to be considered would be to monitor the individual protein permeation obtained for these two MF processes to ensure that β-lactoglobulin and α-lactalbumin are not completely lost during this operation. Therefore, MF permeates should be analyzed by size exclusion high performance liquid chromatography (SE-HPLC). WPC's owe most of their functional properties to these proteins, followed by BSA, Ig, proteose-peptones, lysozyme and finally lactoferrin. In addition, SE-HPLC could be used to monitor and compare the removal of phospholipoproteins during microfiltration of untreated and calcium chloride treated whey. These residual whey lipids continue to be the cause of membrane fouling during whey processing and also have a detrimental effect on WPC's, since they greatly affect their functional properties (Joseph and Mangino, 1988).

Rinn et al. (1990) had previously studied several pretreatment modifications for cheese whey and found that WPC's made from calcium chloride treated whey and processed with a 0.6 μm pore size microfilter had the best foaming and gelation properties when compared to nine other pretreatments. For future research, it would be advisable to study functional properties of WPC's produced with untreated whey and the two-phase cross-flow mode of operation, since some foaming problems were encountered during

Table 19. Effect of Whey Pretreatment on the UF Permeate Flux (L/m^2hr).

Whey Effect		
RW	EW	CW
15.9091[b]	14.9398[c]	19.6693[a]

LSmeans with different letters differ ($p<0.05$)

Table 20. Effect of Air on the UF Permeate Flux (L/m^2hr).

Air Effect	
0%	20%
15.2006[b]	18.4783[a]

LSmeans with different letters differ ($p<0.05$)

Table 21. Interaction between Whey and Air Main Effect on the UF Permeate Flux (L/m^2hr).

Air (%)	MF Permeates		
	RW	EW	CW
0	13.5786[d]	13.3300[d]	18.6930[b]
20	18.2397[b]	16.5496[c]	20.6456[a]

LSmeans in the same row or column with different letters differ ($p<0.05$)

our research. As stated by Harper (1991), multiple functional properties are usually necessary for most food systems, however foaming is not required for applications such as beverages, infant formulas, reformed meats, salad dressings and yoghurt. If foaming during our processing reduced future foaming capacity and stability of the most important whey proteins (β-lactoglobulin and α-lactalbumin), specific applications should be determined for these WPC's. However, a study made by Phillips et al. (1995), revealed that the unfolding of β-lactoglobulin during foaming is reversible after this protein has been subjected to shear and heat. According to Bellara et al (1996), current studies have shown that gas sparging does not deteriorate enzyme activity either. Nevertheless, further studies are suggested in relation to the whey protein configuration and the use of air.

From a processing perspective, there should be a gas liquid separator when processing with air, as suggested by Bellara el at (1996). Proteins in the foam could be recovered in the separator, since the foam collapses.

2. Volume Reduction.

The objective of this experiment was to concentrate the whey proteins. For this purpose 20 L of MF permeate from RW were processed in the ultrafilter using the same operating parameters used throughout our research. To obtain a VCR of at least 20, the process lasted from three to five hours. Since the goal

of this research was not to produce WPCs, but to concentrate proteins to a greater extent, only raw whey MF permeate was chosen for this purpose. Figure 20 shows the relationship between permeate flux and VCR for this particular case. The protein concentration in the UF retentate was increased by a factor of 6 when compared to the initial protein concentration in the feed. The retentate volume was 5% of the initial feed volume, meaning that a VR of 95% was achieved (VCR=20). To obtain WPCs with a protein concentration of 75% or more, several diafiltration processes with distilled water are usually necessary to wash out most of the lactose still present in the UF retentate, allowing further protein concentration. Finally, UF retentates should be spray dried.

The protein losses during MF experiments reflected as low protein permeation values would affect the final protein concentration of WPCs. It would be necessary to find potential uses for MF retentate fractions with high levels of proteins such as BSA and Ig, as revealed by SDS-PAGE. At the same time, further study is needed to optimize the operating parameters for the microfiltration process, in particular, temperature, flow rate and pressure, as well as MF membrane pore size and type to reduce protein losses to those caused by the removal of lipoproteins. According to Karleskind et al. (1995),who studied the protein concentration of WPC's produced by calcium chloride pretreatment of Swiss cheese whey followed by MF, better permeation ratios of α-lactalbumin were obtained when using a 0.1μm polysulphone MF

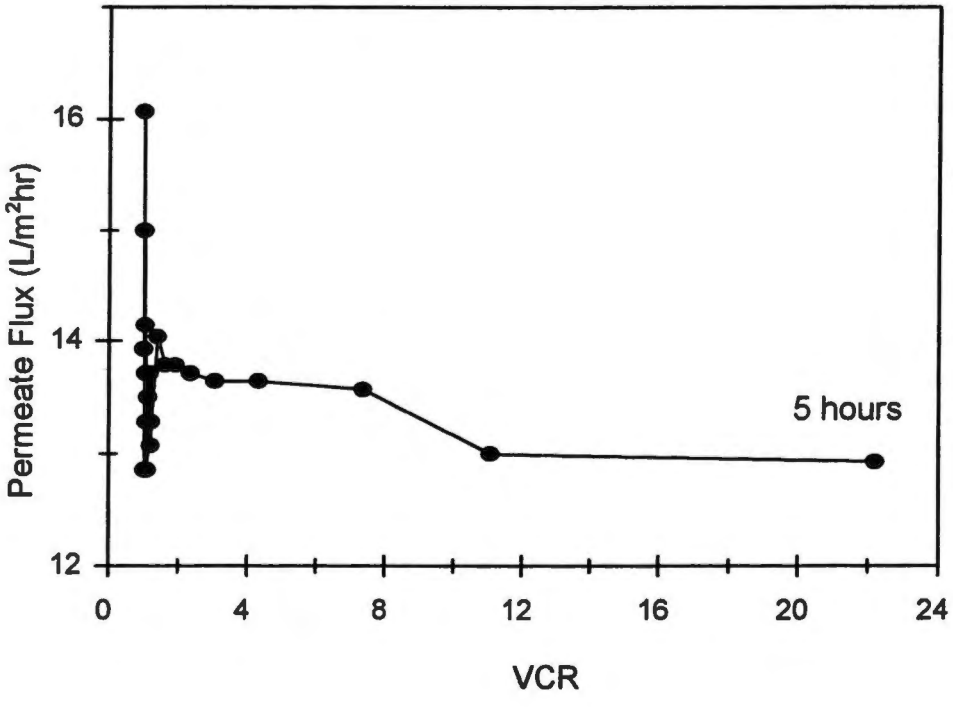

Figure 20. Relationship between VCR and UF Permeate Flux for Raw Whey MF Permeate.

membrane while better permeation rates for β-lactoglobulin were achieved with a 0.45μm membrane. These results do not agree with those obtained by Knopp (1992), who found better permeation rates for α-lactalbumin when processing Swiss cheese whey with a 0.45μm pore ceramic membrane.

3. Individual Proteins by SDS-PAGE.

A 15% polyacrylamide gel loaded with samples of UF retentates from the pretreatment modifications, processed with and without the application of air, showed mainly the presence of α-lactalbumin and β-lactoglobulin. The bands of these proteins appear very dark, as a consequence of the concentration in the UF samples, especially for the retentate samples obtained from processing MF permeates of raw and calcium chloride treated whey. As previously observed during the MF experiments, the bands of BSA and Ig appeared very diffuse on this gel, or were totally absent. Protein bands corresponding to BSA, Ig and other minor proteins present in raw whey were observed in the UF retentate samples from the MF permeate of CW, with and without air. Nevertheless, these gels should be interpreted with caution, since protein permeation should increase with time and because the samples were only representative of the first hour of ultrafiltration. In addition, since the total protein concentration in the samples had decreased as a consequence of the proteins lost during the microfiltration experiments, the stained gels were not as clear as expected.

Although SDS-PAGE can be used to monitor changes in the concentration of individual whey proteins from the whey pretreatments, SE-HPLC analysis of UF retentates and permeates is encouraged. In addition to giving information regarding the protein concentration in the samples, it would confirm whether the UF membrane chosen for this research effectively rejected the whey proteins.

SUMMARY AND CONCLUSIONS

The main objective of this study was to increase permeate flux during MF and UF of cottage cheese whey by applying chemical pretreatments and/or two phase cross flow MF and UF. For this purpose, three different pretreatments were included (1) unclarified whey was used as untreated whey; (2) whey pretreated with EDTA; (3) whey pretreated with calcium chloride where proteins were subjected to thermocalcic aggregation. During the microfiltration experiments our main objective was accomplished by finding the best combination of these chemical pretreatments, flow rate and air percentage. The model used for the statistical analysis was significant ($p<0.05$) and showed the highest permeate fluxes for CW and EW at 10 L/min, with injection of 20% air. Significant differences ($p<0.05$) were found among MF flux of RW when 10% or 20% air was used. There was no significant difference ($p<0.05$) between application of 10% or 20% air, showing that only a small amount of gas sparging is necessary to disrupt the concentration polarisation layer formed by whey proteins. Contrast statements did also reveal that this variable was significant for all pretreatments during the MF experiments. At the same time no significant flux enhancements were obtained when RW was only treated with EDTA.

In the UF experiments, all MF permeates were used as feed solutions with the objective of increasing the UF flux. There were three types of whey and two levels of air, from which the statistical analysis showed the highest flux

enhancement for permeates from CW and when 20% air was introduced in the system, nevertheless, a significant difference in UF flux was obtained for all feed solutions after applying 20% air in the ultrafilter. There was not a significant difference in processing permeates from calcium chloride treated whey (no air) and permeates from raw whey when using the two-phase cross-flow mode of operation. This is important from an economical standpoint, because chemical pretreatment of whey could be minimized during WPC production.

Our secondary objective was to detect differences in protein permeation for all chemical pretreatments, with and without the application of air during the microfiltration experiments. This objective was based on previous reports of protein losses obtained during MF of whey in an attempt to produce delipidized whey protein concentrates. Our results showed significant increases in protein permeation every time that air was introduced in the microfilter. In addition, significantly higher ($p<0.05$) protein permeation was obtained after one hour of processing for permeate samples of raw whey processed by the two-phase cross-flow mode of operation as compared to calcium chloride treated whey (no air).

Further research is needed to optimize operating parameters during the microfiltration process to reduce protein losses only to those due to removal of lipoproteins. At the same time, it becomes necessary to find uses for MF retentate fractions with high concentrations of BSA and Ig, since BSA is a sheet forming protein involved in fouling caused by irreversible concentration.

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APPENDICES

APPENDIX A

Appendix A-1: Analysis of variance of MF permeate fluxes for the combination of whey, flow rate and air.

The MIXED Procedure

Class Level Information

Class	Levels	Values
REP	2	1 2
WHEY	3	cacl2 edta raw
FLOW	5	5 10 20 9.8 9.9
AIR	3	0 10 20

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	39.73030718	
1	2	28.00938490	0.23864316
2	1	27.56725837	0.00357871
3	2	27.53061632	0.00031787
4	1	27.52602455	0.00000200
5	1	27.52599695	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Group	Estimate
REP		0.00000000
DIAG	WHEY cacl2	57.02600000
DIAG	WHEY edta	0.19300625
DIAG	WHEY raw	4.47328358

Model Fitting Information for MFLUX

Description	Value
Observations	18.0000
Res Log Likelihood	-22.0334
Akaike's Information Criterion	-26.0334
Schwarz's Bayesian Criterion	-26.4279
-2 Res Log Likelihood	44.0669

The SAS System

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
WHEY*FLOW*AIR	8	8	34.42	0.0001

Appendix A-2: Contrasts statements results for whey, air, flow main effects and their interactions for the microfiltration experiments.

CONTRAST Statement Results

Source	NDF	DDF	F	Pr > F
Whey Main Effect	2	8	5.06	0.0380
Air Main Effect	1	8	6.01	0.0399
Whey*Air	2	8	0.02	0.9778
Flow Main Effect	2	8	6.90	0.0181
Raw flow=10 Air ME	2	8	5.21	0.0356
Rf=10 Air 0-(10+20)	1	8	10.39	0.0122

Appendix A-3: Analysis of variance of protein permeation for three levels of whey and two levels of air during microfiltration experiments.

The SAS System

The MIXED Procedure

Class Level Information

Class	Levels	Values
REP	2	1 2
WHEY	3	cacl2 edta raw
AIR	2	0 20

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-169.6525465	
1	3	-180.1385124	0.00001947
2	1	-180.1403201	0.00000005
3	1	-180.1403242	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
REP	0.00000000
REP*WHEY*AIR	0.00002060
Residual	0.00000497

Model Fitting Information for PRO

Description	Value
Observations	24.0000
Res Log Likelihood	73.5293
Akaike's Information Criterion	70.5293
Schwarz's Bayesian Criterion	69.1937
-2 Res Log Likelihood	-147.059

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
WHEY	2	5	1004.54	0.0001
AIR	1	5	3334.11	0.0001
WHEY*AIR	2	5	213.72	0.0001

Appendix A-4: Analysis of variance of UF permeate fluxes for three levels of whey and two levels of air.

The SAS System

The MIXED Procedure

Class Level Information

Class	Levels	Values
REP	2	1 2
WHEY	3	cacl2 edta raw
AIR	2	0 20

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
Residual	1.32586058

Model Fitting Information for FLUX

Description	Value
Observations	176.0000
Res Log Likelihood	-275.297
Akaike's Information Criterion	-276.297
Schwarz's Bayesian Criterion	-277.865
-2 Res Log Likelihood	550.5940

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
WHEY	2	170	255.55	0.0001
AIR	1	170	348.52	0.0001
WHEY*AIR	2	170	20.89	0.0001

APPENDIX B

Appendix B-1: LSmeans of the microfiltration permeate fluxes from the combined effect of whey, flow rate and air percentage.

---------- _ADJUST_=LSD(.05) BYGROUP=1 Effect=WHEY*FLOW*AIR ----------

OBS	WHEY	FLOW	AIR	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1	cacl2	9.9	0	23.30000000	5.33975655	8	4.36	0.0024	ABC
2	cacl2	9.9	20	29.96000000	5.33975655	8	5.61	0.0005	A
3	edta	9.8	0	13.44000000	0.31064952	8	43.26	0.0001	C
4	edta	9.8	20	19.96250000	0.31064952	8	64.26	0.0001	A
5	raw	5	0	7.72333333	1.49554063	8	5.16	0.0009	D
6	raw	10	0	11.76000000	1.49554063	8	7.86	0.0001	CD
7	raw	10	10	17.50166667	1.49554063	8	11.70	0.0001	AB
8	raw	10	20	17.82666667	1.49554063	8	11.92	0.0001	AB
9	raw	20	0	15.57850000	1.49554063	8	10.42	0.0001	BC

Appendix B-2: LSmeans of protein permeation during microfiltration for whey main effect, air main effect and interaction whey*air from three levels of whey and two levels of air.

---------- _ADJUST_=LSD(.05) BYGROUP=1 Effect=WHEY ----------

OBS	WHEY	AIR	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1	cacl2		0.64431250	0.00240197	5	268.24	0.0001	A
2	edta		0.54041250	0.00240197	5	224.99	0.0001	B
3	raw		0.49597500	0.00240197	5	206.49	0.0001	C

---------- _ADJUST_=LSD(.05) BYGROUP=2 Effect=AIR ----------

OBS	WHEY	AIR	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
4		0	0.48015833	0.00196120	5	244.83	0.0001	B
5		20	0.64030833	0.00196120	5	326.49	0.0001	A

---------- _ADJUST_=LSD(.05) BYGROUP=3 Effect=WHEY*AIR ----------

OBS	WHEY	AIR	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
6	cacl2	0	0.52380000	0.00339690	5	154.20	0.0001	D
7	cacl2	20	0.76482500	0.00339690	5	225.15	0.0001	A
8	edta	0	0.47797500	0.00339690	5	140.71	0.0001	E
9	edta	20	0.60285000	0.00339690	5	177.47	0.0001	B
10	raw	0	0.43870000	0.00339690	5	129.15	0.0001	F
11	raw	20	0.55325000	0.00339690	5	162.87	0.0001	C

Appendix B-3: LSmeans of the ultrafiltration permeate fluxes for whey main effect, air main effect and the interaction whey*air from three levels of whey and two levels of air.

---------- _ADJUST_=LSD(.05) BYGROUP=1 Effect=WHEY ----------

OBS	WHEY	AIR	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1	cacl2		19.66933424	0.15738429	170	124.98	0.0001	A
2	edta		14.93981481	0.15819338	170	94.44	0.0001	C
3	raw		15.90914931	0.13987727	170	113.74	0.0001	B

---------- _ADJUST_=LSD(.05) BYGROUP=2 Effect=AIR ----------

OBS	WHEY	AIR	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
4		0	15.20055153	0.12713519	170	119.56	0.0001	B
5		20	18.47831404	0.12109328	170	152.60	0.0001	A

---------- _ADJUST_=LSD(.05) BYGROUP=3 Effect=WHEY*AIR ----------

OBS	WHEY	AIR	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
6	cacl2	0	18.69304348	0.24009605	170	77.86	0.0001	B
7	cacl2	20	20.64562500	0.20355133	170	101.43	0.0001	A
8	edta	0	13.33000000	0.22581992	170	59.03	0.0001	D
9	edta	20	16.54962963	0.22159862	170	74.68	0.0001	C
10	raw	0	13.57861111	0.19191003	170	70.76	0.0001	D
11	raw	20	18.23968750	0.20355133	170	89.61	0.0001	B

VITA

Aminta Martínez Hermosilla was born in Valparaíso, Chile. She spent the majority of her life in Quilpué, where she attended elementary school and high school. She entered the Catholic University of Valparaíso where she completed Food Engineering in 1986. In 1987, she moved to Rio de Janeiro, Brasil, with her husband and son but returned to her country in 1991 to work as a professor of Food Engineering at Zipter Institute. In 1993, she came to Raleigh, North Carolina with her husband and her three children. After moving to Knoxville, she entered the Master's program at the University of Tennessee. Her Master's degree was received May 1999.