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STUDIES OF THE REJECTIONS OF MINERALS DURING THE ULTRAFILTRATION OF COTTAGE CHEESE WHEY

BY

DARRYL A. ROEHL 

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

IN

FOOD ENGINEERING

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EDMONTON , ALBERTA

SPRING 1990

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THE UNDERSIGNED CERTIFY THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED STUDIES OF THE REJECTIONS OF MINERALS DURING THE ULTRAFILTRATION OF COTTAGE CHEESE WHEY

SUBMITTED BY DARRYL A. ROEHL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (FOOD SCIENCE) IN FOOD ENGINEERING.

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ABSTRACT

The partitioning of minerals (particularly calcium) between retentate and permeate fractions during the ultrafiltration (UF) of cottage cheese whey was investigated using two different UF systems. The effectiveness of heat as a pretreatment to improve the calcium retention in whey protein concentrates was limited by the overall system performance. In comparison to soluble whey protein systems UF of heat precipitated proteins resulted in lower flux rates for a given applied transmembrane pressure in operating conditions of 200 - 1100 kPa and 20 °C. Precipitated whey proteins caused a more severe decrease in permeate flux with increased protein content and lower achievable final protein concentration than identical soluble whey protein retentates due to rapid increases in viscosity and high uncontrollable module pressures. However, significant increases in transmembrane pressure resulted in increased retention of Ca, P and lactose.

Mathematical expressions, defining response surfaces in multidimensional space, were developed for the pilot scale ultrafiltration of cottage cheese whey to correlate flux decline and the partitioning of minerals (Ca, Na, P, Mg,

K) between retentate and permeate fractions as functions of process conditions. A 5 variable (pH, temperature, flowrate, average transmembrane pressure, EDTA addition) 5 level, central composite experimental design produced empirical models that adequately described experimental observations (R^2> 0.80). and indicated a variable response of microsolute rejections to changes in UF processing conditions, particularly pH and sequestrant addition using a plate and frame UF system with polysulphone membranes. Divalent cations (Ca,Mg) typically exhibited more positive rejections than monovalent cations (Na, K) over the experimental range. Calcium rejections ranged from a minimum of -3% at pH 4.5 and 0.35 g/L EDTA to a maximum of 45% at pH 7.0 and 0.0 g/L EDTA addition. For identical experimental conditions, observed sodium rejections were -33% and 21% respectively. Flux decline was faster as the pH increased, but increasing sequestrant addition retarded the rate of flux decline. Similar effects were observed with a tubular metallic membrane system, in which increasing pH and temperature resulted in reduced flux. Calcium retentions ranged from a minimum of -18% at pH 4.5 and 0.0 g/L EDTA to a maximum of 78% at pH 7.0 and 0.20 g/L EDTA addition and were affected primarily by pH. The

addition of EDTA did not improve UF performance.

Three whey protein concentrates (WPC) prepared by UF of cottage cheese whey adjusted to pH 3.9, 4.5 and 6.3 were incorporated into 4% cottage cheese creaming mixes as a substitute for a significant proportion of whole milk. Dressed curds were evaluated for appearance, flavor and overall acceptability by an untrained taste panel. No significant differences were found between the WPC containing formulations and control samples at preparation and after 7 days storage. The pH of the dressings, and dressed curds were influenced by WPC substitution.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I. INTRODUCTION

I. 1 - Ultrafiltration in Dairy Processing

Ultrafiltration (UF) is a membrane process used for concentration and fractionation of emulsified, colloidal and dissolved substances in aqueous solutions. Ultrafiltration is employed commercially in the dairy industry for soft and semi-soft cheese manufacture, and for fractionation of whole milk, skim milk, buttermilk and sweet whey (Kosikowski, 1986). The application of UF for on-farm milk concentration (Jameson, 1983), milk protein standardization and hard cheese manufacture is currently being actively investigated.

Whey is the liquid byproduct of milk drained from precipitated casein during the manufacture of cheese or industrial casein. There are two types of whey: sweet whey from Cheddar, Swiss, American style cheeses and rennet casein, and acid whey from cottage cheese (Wong et al. 1976), bakers cheese, quarg (Jelen and Renz-Schauen, 1989) and acid casein manufacture. In either case, the whey constitutes 85 -90% of the original milk volume. Although whey is an excellent source of nutrients, its low total solids content and the types of nutrients present

have hindered its profitable utilization. Ultrafiltration combined with an efficient large scale evaporation and drying technology has resulted in the commercial availability of a wide range of whey based products. Acid whey, because of its high mineral content and the additional processing and nutritional constraints this has brought, has remained chronically underutilized.

The high levels of calcium (Ca) and other minerals in acid whey have proven detrimental to effective UF performance (Matthews et al., 1978). The mineral content of whey protein concentrates (WPC) effects their functionality and suitability for use as a dietary supplement. The high levels of sodium (Na), and potassium (K) found in acid whey are not desirable nutritionally whereas the presence of calcium is a primary determinant of whey protein functionality. Reduction of total mineral contents of WPC products to tailor their functionality for specific uses is presently achieved by diafiltration and electrodialysis.

Calcium, an essential mineral of human dietary needs is found in significant quantities in milk and whey.

Its contribution to the prevention of osteoporosis and hypertension is only now being understood (Jagerstad, 1982, McCarron et al., 1982). Increasing public education concerning appropriate consumption of dietary calcium has led to current high demand for calcium containing foods. This, coupled with a growing trend on the part of consumers to prefer food supplements and ingredients used in their "nature identical" forms results in acid whey being potentially valuable as a source of naturally occurring and nutritionally available calcium.

The ability to recover milk calcium in the form of high calcium WPC to fortify other products would be a highly marketable end use for whey. If combined with the selective removal of less desirable minerals in whey, both the nutritional and functional properties of the WPC might be tailored.

Traditionally the use of ultrafiltration in the dairy industry has been applied to the concentration and fractionation of macromolecules. It may be expected that any pretreatment of whey which effects the nature of the interactions between membrane impermeable whey proteins

and the mineral constituents would effect the partitioning of mineral species between the two UF fractions. Heat denaturation of whey proteins in the presence of calcium may result in the association of whey proteins and calcium to form precipitated aggregates (Morr and Josephson, 1968). Such specific interaction might be utilized as a UF pretreatment if the precipitated whey proteins could be effectively ultrafiltered. Although heat pretreatment of acid whey prior to ultrafiltration has been investigated to enhance performance (Hayes et al., 1974 , Buhler et al., 1981), ultrafiltration of heat precipitated whey proteins from acid whey has not been fully explored.

Little attention has been paid to the partitioning of minerals during ultrafiltration other than recognition of their effect on ultrafiltration processing. Despite the references in literature linking the importance of Ca to the effectiveness of whey ultrafiltration and the functionality of whey protein concentrates, information describing the rejections of minerals during ultrafiltration of whey has not been found.

The effectiveness of pH reduction and sequestrant addition as means of improving the UF performance of acid whey has been reported (Patocka and Jelen, 1987). Explanation for the effectiveness of these treatments center on the complete solubilization of calcium phosphate at low pH, and the competition between sequestrant, protein and membrane surfaces for free Ca ions.

Cottage cheese whey remains a significant, presently underutilized byproduct of the North American dairy industry due, in large part, to its high mineral content. To maximize the full potential of cottage cheese whey as a source of valuable proteins and minerals, the understanding of partitioning patterns for specific minerals between retentate and permeate fractions is necessary.

I.2 - Objectives

The objective of the research was to determine the effect of selected UF systems, operating parameters and whey pretreatments on the observed partitioning of minerals between the concentrate and permeate fractions in order to

maximize calcium retention while preserving overall UF performance. An objective of this work was to determine if mineral species exhibit individual rejections characteristics, which may be influenced by various pretreatments or system parameters.

Ultrafiltration of heat precipitated whey proteins was investigated for its potential to increase calcium levels in the concentrate, subject to the feasibility of ultrafiltration of heat precipitated whey proteins.

Ultrafiltration experiments with cottage cheese whey were replicated on two separate membrane systems to determine performance and mineral rejections independent of systematic error.

The original impetus for this work was industry based, stemming from the desire for an all natural, calcium enhanced creamed cottage cheese. The suitability of liquid whey UF protein concentrate substitution for milk in cottage cheese dressing was used in the investigations as a model for other possibilities of whey protein concentrate supplementation in dairy products.

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Chapter II - LITERATURE REVIEW

II - 1 Ultrafiltration of Cottage Cheese Whey

II - 1.1 Whey

The production of whey in Alberta was most recently reviewed by Danish Turnkey Dairies (1985). From cheese and cottage cheese manufacture, whey production in Alberta is in the range of 510 tonnes/day from 15 dairies. Although whey production is concentrated in the north central and southern region, a significant proportion (approx. 30%) is produced in smaller quantities in other regions. The distribution of whey utilization by regions is shown in Table II - 1. Overall, approximately 70% is transported and dumped on fields, 25% is treated by municipal sewage systems, and the remaining 5% utilized as animal fodder. The cost and difficulty of disposal, and the lost value of the potentially functional products make whey disposal a significant problem. This problem is compounded for those plants whose main avenue of disposal is a municipal sewage system.

An Edmonton area dairy, processing 300,000 kg of fluid milk per day, with whey production of approximately

20,000 Kg per day solely from cottage cheese presents a problem typical of Alberta whey producers. Although costs of whey disposal, by discharge to the sewer, are in excess of $25,000 per year, the relatively small quantities of whey produced are not conducive to viable economic solutions for in-house processing and solids recovery. However, this sewage discharge represents a pollution load equivalent of 28,000 persons (Danish Turnkey Dairies, 1985). Increasing constraints of limited municipal sewage treatment capacity may soon result in required in-plant whey processing to meet municipal guidelines for disposal.

II - 1.2 Whey Composition

The compositions of acid and sweet whey powders are summarized in Table II - 2. Fluid whey contains about 6.5%, 4.9% and 0.8% total solids, lactose and protein respectively. The major determining factors of whey composition are defined by the cheese manufacturing process (Adrian and Bourlier, 1980).

II - 1.3 Minerals in Whey

It is generally true that all minerals in milk are present in whey with some differences in contents due to the binding of minerals by casein during coagulation. A summary of mineral contents of various wheys is shown in Table II -3. When comparing the mineral content of the wheys it is important to note the pH of the whey. The pH at the separation of curds and whey (dipping) affects whey composition (Hill et al., 1982) and largely determines the mineral compositions of sweet and acid wheys. Acid whey due to the lower pH contains more lactic acid, calcium, phosphorus, magnesium, zinc, iron, and copper than sweet whey (Adrian and Bourlier, 1980).

Distribution of minerals between cheese and whey are affected by the solubilization of colloidal minerals at reduced pH. Subsequent losses of minerals due to curd washing are substantial. Solubilized minerals are leached from the casein curd. Using less wash water increases the mineral content of the curd but does not significantly affect the levels of phosphorus due to its colloidal nature. As an example, the calcium content of cottage

cheese is about 30mg/100g (Wong et al., 1976).
In whey , which is basically free of casein, any mineral-protein interactions will be defined by the association of minerals to the whey proteins.

II - 1.4 Whey Proteins

Whey proteins can be described as the water soluble proteins remaining in the supernatant of skimmilk after acidification to pH 4.6. Most are globular and are heat labile such as Beta -lactoglobulin, alpha-lactalbumin, bovine serum albumin (BSA) and immunoglobulins.

In addition, whey includes proteolytic fragments of caseins (Walstra and Jenness, 1984) which make up the proteose peptone fraction. The proteose peptone fractions are those proteins which remain soluble after heating, but which precipitate upon addition of 12% w/v trichloroacetic acid (TCA) (Greig, 1979). The proteins of whey and some of their properties are summarized in Table II -4. It has been observed that whey proteins at concentrations in excess of approximately 0.7% stabilize calcium salts from

precipitation (Brule et al., 1978).

It is expected that the proteins in solutions with pH above their isoelectric point will be in a net anionic form, and will act as counterions for the binding of cations. Such an interaction is based on the non-specific association of charged species to balance net charge. The various proteins in whey also exhibit specific binding affinities for metallic cations, particularly calcium.

Beta-lactoglobulin is the most abundant serum protein accounting for up to 50% of the non-casein proteins in milk. It exists as a dimer at pH 5.2 but dissociates to monomers below this pH. Although quantitatively the most important whey protein, the calcium binding characteristics of Beta-lactoglobulin have not been thoroughly investigated. The work of Zittle et al. (1957) indicated a close relationship between calcium and Beta-lactoglobulin, but did not quantitate the relationship. The only estimate quantifying the relationship between calcium and Beta-lactoglobulin is approximately 15 moles Calcium / mole of protein at pH 6.8 (Zittle et al., 1957).

Serum albumin (SA) of bovine origin is a globular protein of high molecular weight (69,000). SA was estimated as having up to 10 binding sites for Ca per mole of protein in the presence of NaCl (Edsall et al. 1950). For a $CaCl_2$ containing solution, it was estimated that no binding sites should exist for calcium on the BSA with a net protein charge of +20, and that 8 sites would exist in conditions producing a net protein charge of -20. However, it was speculated that the total number of binding sites was considerably higher than 10. In a high ionic strength solution and at low pH, the data of Edsall et al. (1950) indicated that several moles of calcium remained bound to the protein despite the unfavorable conditions.

Significant salt binding to serum albumin occurs in the presence of calcium dichloride and magnesium dichloride due primarily to the salting-in effect of these salts (Arakawa and Timasheff, 1982). It was estimated that SA binds 23.3 moles Ca per mole of protein at pH 5.6 in a 1 M calcium chloride solution.

Alpha lactalbumin has a relatively low molecular weight of

approximately 14,000 D; at pH below its isoelectric point (4.2 - 4.5) this protein forms dimers and trimers. Its ability to associate and aggregate is dependent on ionic strength and protein concentration (Merin, 1979). Alpha-lactalbumin has been shown to be a calcium metalloprotein (Hiraoka et al., 1980) containing a strong cation (calcium) binding site (Murakami et al., 1982). The binding of the metal ion was found to induce a conformational change in the protein. In addition, it was speculated that up to 3 additional weak binding sites for divalent calcium exist. Two calcium binding sites on apo-bovine lactalbumin with pKa's of 5.56 and 3.5 has been reported (Kronman et al., 1981).

The high degree of calcium metallization of alpha-lactalbumin was observed by atomic absorption in commercial "pure" protein preparations which may contain up to 23% calcium/mole (Murakami et al., 1982). In the presence of other cations, lactalbumin was observed to have preferential binding for Ca (Murakami et al., 1982).

II - 1.5 Heat Induced Whey Protein Reactions

Protein aggregation in heated whey systems is a multi-reaction process involving protein denaturation through thiol-disulfide group, hydrogen, and hydrophobic bond reactions, aggregation to intermediate sized particles through thiol-disulfide group reactions, and gross aggregation of protein particles in the presence of calcium ions (Morr and Josephson, 1968). Calcium promotes the aggregation of Beta-lacotglobulin (Varunsatian et al., 1983). The open protein structure during heat treatment permits formation of a protein-calcium complex (de Rham and Chanton, 1984). Calcium bridges act as a stabilizing factor for protein clusters after cooling.

While it is known that whey proteins are more resistant against heat induced coagulation at low pH (Jelen and Buchheim, 1984), interaction between calcium and proteins upon heating remains an important step in protein coagulation. The high heating of whey significantly alters the relationship between calcium and whey proteins as a result of the calcium mediated flocculation of protein clusters upon cooling. It would appear possible

to specifically increase the levels of calcium in a whey protein concentrate by heating to the point of complete denaturation, followed by cooling to allow precipitation and finally ultrafiltration. The ultrafiltration of high heat treated cottage cheese whey is not at present a commercial process and relevant technical information in literature is scarce. The practicality of such a pretreatment to improve calcium recovery would depend directly on the feasibility of ultrafiltering heat precipitated whey proteins.

II.2 - Rejection of Minerals During Whey Ultrafiltration

Ultrafiltration in the dairy industry has been applied as a fractionation/concentration unit operation to the separation of macromolecules from dilute solutions. A large amount of information exists describing the interrelationship between operating parameters and ultrafiltration performance (Matthews et al., 1978) measured by permeate flux.

Minerals in milk and whey systems significantly influence UF processing. However, little attention has been paid to

minerals during ultrafiltration other than to recognize the nutritional and functional problems caused by high salt contents in UF whey concentrates (De Wit, 1981) and to observe the contribution of calcium to the mechanisms of fouling and long term flux decline (Patocka and Jelen, 1987, Nisbet et al., 1981, Ennis et al., 1981). In continuous cheese production utilizing ultrafiltration, defects of bitterness (Jelen and Renz-Schauen , 1989) excessive curd strength and increased buffer capacity (Srilaorkul et al., 1989) have been attributed to high retentions of ash, particularly calcium (Hansen 1981, Maubois, 1980).

Under idealized conditions, ultrafiltration is approximated as a simple sieving process, with the low molecular weight milk constituents passing relatively unhindered through the membrane. Although ultrafiltration membranes are generally assumed not to retain compounds with molecular weights smaller than the membrane cut-off, they often display a selectivity towards species significantly smaller than the nominal pore size of the membrane. Retention of small color compounds during the membrane processing of enzymes has been observed (Kerkhof,

1988). Ultrafilters which normally show no significant rejection for amino acids and salts do so in the UF of skim milk (Matthews et al., 1978, Walsh et al., 1988) and whey (Peri et al., 1973).

Some of the reports on positive rejections of ash or mineral constituents in UF of milk (Yan et al., 1979, De Boer et al., 1973, Pompei et al., 1973, Brule et al., 1974, Peri et al., 1973, Brule and Fauquant, 1981, Kulozik, 1988, Hansen, 1981) and whey (Matthews et al., 1978, Hiddink et al., 1978, Kiviniemi, 1977) indicate possible mechanisms of mineral retention.

The reasons for less than 100% passage of some mineral species is due not to the membrane itself, but to chemical interactions between low molecular weight and high molecular weight substances and conditions of concentration polarization. Minerals associated with UF-impermeable macromolecules, bound either directly or in colloidal structures would be expected to be retained at a rate proportional to the retention of the macromolecule. Conditions which did not disturb these associations will result in a final mineral concentration no less than that

expected from the complete retention of this mineral fraction.

Hiddink et al. (1978) observed that during the ultrafiltration and diafiltration of whey and buttermilk, pH could be used to influence the ash content and composition of the retentates. Lowering the pH of milk before ultrafiltration causes greater amounts of colloidal or protein bound calcium, magnesium, and phosphate to be molecularily dispersed (Hiddink et al., 1978) and removed during ultrafiltration. The observations that at pH 6.6 (above the isoelectric point of whey proteins) anions were preferentially removed while the same is true for cations such as Na, Ca and K at pH 3.2 was explained by the theory of the Donnan effect (Hwang and Kammermeyer, 1975, Hiddink et al., 1978). These observations agreed with calculated net charges on the whey proteins acting as impermeable counterions for minerals. The results from this experiment indicated that cation rejections changed with a change in the pH of the whey, and indicated that specific cation rejections may be different for identical experimental conditions.

The use of macromolecular complexes for the selective UF concentration of minerals has been investigated (Strathman and Kock, 1978). Ultrafiltration can be effectively applied for selective mineral concentrations when combined with a water soluble macromolecular complex which selectively binds metal ions or groups of ions. Permeable ions will be selectively retained along with the retention of the impermeable counterions. Concentration of the macromolecules selectively enriches the concentration of the associated microsolutes. Any chemical modification of the system which influences the degree of association between the macromolecule and metal, will alter the retention of the metal in the retentate. In acid whey, the whey proteins present would act as the impermeable counterions. As previously discussed, whey proteins have shown to exhibit specific affinities for calcium. The extent to which this would effect calcium rejection has not been previously reported in literature.

When a gel forming macromolecule is filtered using a membrane of sufficiently small pore size to retain the majority of the macromolecule, the phenomenon of concentration polarization produces a dynamic membrane on

the surface of the ultrafilter (De Wit, 1981, Tanny, 1978, Kerkhof, 1988, Nakao et al., 1979). A dynamically formed membrane composed of a complex mixture of whey constituents (particularly proteins) is expected to mask the rejection characteristics of the original membrane. The increased retentions of color compounds during enzyme microfiltration (Kerkhof, 1988) have been postulated to be due to these compounds having to diffuse through a deposited protein matrix. For the ultrafiltration of blood plasma using a cellulose acetate membrane system, chloride ions and end metabolic products were observed to pass through a stable protein gel in preference to Ca, Na, and K ions (Dorson et al, 1971). In the case of milk, the gel layer of protein (and fat) produced during milk UF has been characterized as a series resistance for the transport of microsolutes (Yan et al., 1979).

The deposition of gel-forming polymers and polyelectrolytes on an ultrafilter may be characterized as a Class I dynamic membrane (Tanny, 1978). The gradient of concentration is the net result of the convection, and back diffusion of macromolecules to and from the surface of the membrane. If the concentration of the

macromolecule at the membrane reaches a gel concentration, this layer will continue to grow until balanced by back diffusion, and will act as an additional membrane in series with the UF membrane.

Solute-membrane interactions (Fane et al., 1983) in the form of protein adsorption, caused by reversible and irreversible hydrogen, hydrophobic, ionic and electrostatic bonds will produce a protein-membrane composite with rejection characteristics and flux characteristics substantially different from the membrane. Although the rejections of individual minerals were not investigated, several observations on the nature of proteins absorbed to the membranes were made (Fane et al., 1983). Highest flux was observed to occur when the protein is enlarged and highly charged in conditions of pH extremes in non-ionic solutions. In ionic solutions, the charges are shielded giving a tighter, less permeable deposit. At isoelectric pH, flux remained unchanged by salt addition.

The inherent resistance to transport of minerals at the membrane surface is due to the rejection of the membrane

and associated charged macromolecules at its surface.

Several developments in membrane technology have utilized the rejection characteristics of charged membranes (Bhattacharyya and Cheng, 1986) or dynamically formed polyelectrolyte blend membranes (Akred et al., 1980, Spencer et al.,1984).

Low pressure ultrafiltration with charged anisotropic (thin skin) non-cellulosic membranes has been studied for the separation and concentration of various inorganic salts present in aqueous solutions (Bhattacharyya and Grieves, 1977, Bhattacharyya and Cheng, 1986, Bhattacharyya et al., 1974). The simultaneous achievement of the adequate rejection of inorganic ions and the high rejection of organics has been achieved with charged ultrafiltration membranes for complex wastes (Bhattacharyya and Grieves, 1977). The presence of a highly charged polyelectrolyte layer at the membrane surface significantly affected the rejections of cations during the ultrafiltration of gelatin and salt solutions (Akred et al., 1980).

Highly negative rejections of calcium (up to -400%) and sodium (up to -700%) were measured for the ultrafiltration of gelatin solutions using neutral membranes. The strongest negative rejections were observed for conditions of low solution pH, high gelatin concentration, and low transmembrane pressure. The observations were interpreted on the basis of the Donnan membrane theory. However, in ultrafiltration conditions favoring gel-polarization (high transmembrane pressures) the presence of a positively charged gel layer retarded the passage of cations, leading to positive rejections of calcium ions.

The effect of manipulatable variables of ultrafiltration processes have been studied in great detail. Transmembrane pressure, cross flow velocity, temperature, solution pH, ionic strength, feed composition, feed concentration and sequestrant addition have all been investigated. Such studies have concentrated on process conditions required to maximize membrane flux and minimize membrane fouling. Determination of rejection characteristics of ultrafiltration membranes have dealt primarily with the rejection of macromolecular complexes.

Studies to determine how ash contents of UF concentrates can be reduced indicate that diafiltration and pH reduction (Pompei et al., 1973, Brule et al. 1974, Hiddink et al., 1978) are treatments for reducing total ash content. However, with the exception of Hiddink et al. (1978) and Kiviniemi (1977) little work has been published on whey systems where rejections of individual mineral species have been reported for specifically defined operating conditions. Although literature indicates potential for positive rejections of minerals, and variable response to ultrafiltration conditions, specific information relating operating parameters of ultrafiltration to specific mineral retentions have not been reported. The recovery of calcium during the ultrafiltration of whey has not been studied insofar as could be determined from literature.

II.3 - Calcium And Its Significance in Human Nutrition

Adequate intake of calcium is an essential component of human dietary needs. Meeting nutritional dietary calcium intake requirements has been shown to prevent osteoporosis (Jagerstad, 1982), aveolar bone loss and accompanying oral

health problems, and hypertension (McCarron et al., 1982). The calcium lost to the whey during cheese manufacture represents a significant source of a nutritionally important mineral.

It is known that in cultures with relatively high calcium intake, individuals reject a large proportion (70%) of ingested calcium (Jagerstad, 1982). Calcium absorption in fact adapts to calcium intake in order to maintain calcium balance. Although most adults can adapt to a low calcium intake, preadolescents, pregnant and lactating women, and the elderly are especially sensitive to low levels of dietary calcium.

In addition to high levels of calcium, the presence of several cofactors and enhancers of calcium absorption in dairy products make them particularly suited as a calcium source. As well as being affected by age, endocrine function, nutritional status, calcium requirements of the individual, physical activity, emotional state, use of medications and general health (Jagerstad, 1982) calcium absorption and retention is affected by several dietary components. The contributions

of protein, phosphorus, lactose and vitamin D are important for efficient calcium utilization from milk products.

The presence of lactose in the diet is thought to improve calcium absorption in the intestine. Since lactose is only slowly absorbed by the intestine it is speculated that free sugar in the lower gut may modify the indigenous bacterial flora and produce a lower pH, which is favorable for calcium absorption (Vaughon and Filer, 1960). Some evidence also exist that lactose may chelate calcium to form soluble salts assisting in absorption (Charley and Sultman, 1963). Several authors indicate that lactose can stimulate intestinal absorption and increase retention of calcium in experimental animals and humans. However, a large proportion of the published claims appear to refer to the results of only a few original researchers.

Studies (Wegener and McCarron, 1986, Weaver and Evans, 1986) indicate that appropriate levels of specific cations in relation to one another may be as important as their recommended daily allowances. During the study of hypertension, it was observed that adequate levels of

dietary calcium could offset the increase in blood pressure due to high sodium intake. The implication in these studies was that controlling the relative ratios of specific minerals in the diet was an important factor in mineral nutrition. Of particular interest in these studies was the relationship of calcium, sodium and potassium, the three most abundant cations in cottage cheese whey.

Adjustment of the ratios of these minerals in a whey protein concentrate to meet specific human nutritional guidelines could offset potential nutritional problems caused by a high overall salt content.

Recent consumer demand is for natural or nature identical products. The primary impetus for this study was the desire on the part of a regional dairy to produce a calcium enriched creamed cottage cheese without the use of a non-dairy calcium supplement. Consumer demand for a more nutritionally complete product, and the industrial partner's guidelines on cottage cheese manufacture did not permit the use of commercially available mineral supplements as used in other jurisdictions. The favored

approach was to produce a natural product with a minimum disturbance of the traditional cheesemaking by supplementation of the cottage cheese dressing with the calcium available in the whey. Ultrafiltration was chosen for consideration as it represents a well developed operation for the concentration and fractionation of whey proteins. Although calcium recovery from cheese whey would represent a significant marketing advantage, the commercial success of the calcium enriched whey protein retentate would be determined by the technical and economic feasibility of the ultrafiltration process.

TABLE II-1 Utilisation of Whey In Alberta (Danish Turnkey Dairies, 1985)

	WHEY PROD	SUPPLIED TO FARMS	DUMPED TO FIELDS	DIRECT TO SEWER
	%			
NORTH EAST REGION	13	10	90	N/A
NORTH WEST REGION	8	27	14	59
NORTH CENTRAL REGION	32	N/A	92	8
SOUTH CENTRAL REGION	8	6	N/A	94
SOUTHERN REGION	39	69	31	N/A

TABLE II-2 Composition and Nitrogen Distribution of Acid and Sweet Whey Powders

	Sweet Whey %	Acid Whey %	Reference
TOTAL SOLIDS	96	96	a,b
PROTEIN (N x 6.38)	13	12	a,b
	%Distribution of Protein In Whey		
beta-lactoglobulin	39		c,d
alpha-lactalbumin	15		c,d
beta-CN P + PP-3	14		c,d
Serum albumin	4		c,d
Immunoglobulin	6		c,e
NPN	25		
LACTOSE	72	65	a,b
LACTIC ACID	0.2	4.2	a
ASH	7.8	11.2	a,b

beta-CN P = Proteoses derived from beta-casein
PP-3 =proteose-peptone component 3
a Kosikowski, 1979
b Glass and Hedrick, 1977
c Larson and Rollerie, 1955
d Davies, 1974
e Cerbulis and Farrell, 1975

TABLE II-3. Mineral Contents of Various Wheys and Ultrafiltration (UF) Milk Permeate

	COTTAGE CHEESE WHEY (1)	LACTIC ACID WHEY (2)	RENNET WHEY (2)	UF MILK PERMEATE (3)	SWEET WHEY (4)
	mg/ 100 g				
Calcium (Ca)	108.8	147.9	39.9	40.0	40 -50
Magnesium (Mg)	10.1	13.9	7.8	6.0	8 -10
Sodium (Na)	46.9	62.6	47.0	50.0	36 -51
Potassium (K)	133.0	140.6	143.0	150.0	140 -160
Phosphorus (P)	79.4	90.0	37.4	40.0	40 -55
	ug / 100 g				
Zinc (Zn) (5)	381.3	n.a.	n.a.	n.a.	n.a.
Iron (Fe)	60.8	n.a.	n.a.	n.a.	n.a.
Copper (Cu)	8.5	n.a.	n.a.	n.a.	n.a.
Manganese (Mn)	2.6	n.a.	n.a.	n.a.	n.a.

n.a. - not available

1. Wong et al. (1976)
2. Merin (1979)
3. Brule et al. (1974)
4. Walstra and Jenness, (1984)
5. Wong et al. (1977)

TABLE II-4. Proteins of Whey and Some of Their Properties (Merin, 1979).

PROTEIN	% OF SKIMMILK PROTEINS	pI	MOLECULAR WEIGHT
SERUM ALBUMIN (BOVINE)	0.7 - 1.3	4.7	66,500- 69,000
BETA-LACTOGLOBULIN	7.0 - 12.0	5.3	18,275- 20,000
ALPHA-LACTALBUMIN	2.0 - 5.0	4.2-4.5	14,146- 14,174
IMMUNOGLOBULINS:	1.9 - 3.3	5.6-6.0	
IgG1	1.2 - 3.3		161,000- 163,000
IgG2	0.2 - 0.7		150,000- 154,000
IgA	0.2 - 0.7		385,000- 417,000
IgM	0.1 - 0.7		1,000,000
FREE SECRETORY COMPOUNDS (FSC)	0.2 - 0.3		70,000-96,000
PROTEOSE PEPTONES	2.0 - 6.0		14,300 4,100; 9,900

pI - Isoelectric Point

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CHAPTER III. ULTRAFILTRATION OF HEAT PRECIPITATED WHEY PROTEINS

Introduction

Continued research indicates the need for improvements in processing effectiveness of ultrafiltration (UF) for the recovery of food grade proteins from whey and other protein systems. A study comparing the ultrafiltration performance of soluble and isoelectrically precipitated soya proteins (Hoare and Devereux , 1985) suggested that substantially different flux relationships between transmembrane pressure and protein concentration may be obtained for soluble and precipitated proteins. The resulting potential improvements in UF performance at higher protein concentrations by precipitating the proteins may be important especially for those final products where solubility is not required. For cottage cheese whey ultrafiltration, Tarnawski and Jelen (1985) reported a 30% increase in ultrafiltration permeate flux for whey adjusted to pH 6.5 and boiled for 30 minutes. However, the UF of the heated whey at this pH caused irreversible membrane fouling. Modler and Harwalkar (1981) observed that the heating of sweet whey (90 °C , 15

minutes) when acidified to levels of pH 2.4 to 3.5, did not influence flux rates, while two control wheys at pH 6.5 and 6.2 pasteurized (85 °C, 15 s) or heated (90 °C, 15 minutes) differed significantly. The heated whey flux rate increased by approximately 25% when compared to the pasteurized whey. The differences between the observations in the two pH ranges might be due to the heat stabilization of whey proteins in acid pH (Jelen and Buchheim, 1984).

Heating of HCl casein whey adjusted to pH 5.8 at 85 °C was reported to produce improvement in ultrafiltration performance (Hayes et al., 1974). This treatment may have produced intermediate heat induced protein aggregates that were not large enough to precipitate.

Adjustment to pH 6.1 of untreated of skimmed sweet and acid wheys, and heating to either 95 - 100 °C for 10-30 minutes or to 120-160 °C for 10s - 2 minutes prior to ultrafiltration produced 70% to 300% improvement in permeation rates over soluble whey systems (Buhler et al., 1981).

The use of ultrafiltration with heated whey protein systems might also be useful for the recovery of the "traditional lactalbumin". Yields and effectiveness of centrifugal separation for recovery of heat denatured whey proteins from whey is limited by the degree of denaturation, by the presence of heat stable whey protein fractions and the separation efficiency of the centrifuge. Typically, the recovery of proteins by the Centri-whey process is approximately 70%, while in heat deproteination for lactose crystallization, about 50% of the whey protein was removed by heating (Buhler et al., 1981).

Heating of UF whey protein retentate reduces the solubility of the whey proteins in the isoelectric range (Modler and Harwalkar, 1981), making them potentially easier to recover (Buhler et al., 1981). Recovery of heat precipitated whey protein via ultrafiltration as opposed to centrifugation could increase the yields by the incorporation of the non heat precipitable proteins.

Many sources (Buhler et al., 1981, Hayes et al., 1974, Modler and Harwalkar, 1981, Tarnawski and Jelen, 1985) have indicated that improvements in UF of wheys in the

neutral pH range are possible by pre-heating the whey prior to processing. The information on the effects of heating at conditions typical for acid wheys are scarce. Calcium acts as a mediator for tertiary whey protein aggregation upon heating. Additional associations between Ca and whey protein complexes might result in increased relative retention of Ca in the valuable protein retentate, especially when using the calcium rich cottage cheese whey.

The overall objective of this investigation was to study the ultrafiltration performance of soluble and heat precipitated acid whey protein solutions. Transmembrane-pressure / flux profiles, the flux/protein concentration profiles, viscosity/protein concentration relationships and Ca, P, and lactose retentions for soluble and precipitated whey protein systems were determined.

Materials and Methods

Preliminary UF whey protein retentates were prepared using cottage cheese whey (pH 4.4) provided by a local dairy. The whey was processed using a DDS (De Danske

Sukkerfabrikker, Nakskov, Denmark) Module 35/36 13.5 m^2 pilot scale ultrafiltration unit equipped with GR61-PP polysulphone membranes (MW cutoff 20,000). A 1.2% protein retentate and a 1.2% protein diafiltrate were prepared. The pH range of all the retentates was 4.2-4.4. Aliquots of the original "high lactose" retentate and of the diafiltrate were heated at 90 °C in a steam jacketed vessel with agitation for 30 minutes to produce the heat precipitated whey protein systems. Heating of the UF retentates caused a pH drop of about 0.2 units in all retentates. Some of the soluble and precipitated high lactose whey protein concentrates were diluted with distilled water to produce 0.25% protein solutions. All solutions were stored at 4 °C prior to use and were used within 5 days.

In all experimental trials, fifty litre batches were processed using a DDS Lab-20 ultrafiltration unit equipped with 20-GR61-PP polysulphone membranes (0.36 m^2) and a Rannie (Model 12-18/50, Rannie Co., Copenhagen) triple piston pump with variable speed drive. Volumetric flow rates were accurately controlled. Inlet and outlet pressures were measured by pressure gauges, and the

processing pressure was regulated by a needle valve at the module outlet. Average transmembrane pressure was determined as the average of inlet and outlet pressures.

Each experimental run commenced with thoroughly cleaning the membranes as per manufacturer's instructions. The protein solution to be processed was then placed in a jacketed bulk feed tank and allowed to circulate through the module with full recycle for 30 minutes at the desired feed flow rate and minimum achievable transmembrane pressure. Average transmembrane pressures were then increased, the system allowed to equilibrate for 10 minutes, and flux measurements were taken in duplicate. The experimental pressures were varied between 200 kPa and 1100 kPa.

For the determinations of initial flux as a function of protein content, the product was concentrated in a series of steps with intermittent membrane cleaning. After each cleaning cycle, the system was reequilibrated with the reintroduced protein concentrate for 20-30 minutes before flux measurements were taken and a small sample removed for the determination of viscosity and component analysis.

The procedure was repeated for all datapoints. Average transmembrane pressures of 430 kPa were maintained where possible for all concentration trials.

Protein concentrations were determined using a modified Lowry protein assay with commercial Bio-Rad protein assay reagent, Coomassie Brilliant Blue G-250 (Bio-Rad, Richmond, Calif.). Lyophilized bovine serum albumin (Bio-Rad, Richmond, Calif., Lot 28367) was used as a standard. Mineral contents were determined by Inductively Coupled Plasma (ICP) Emission Spectrophotometry, using a Fisher Atomscan 2000 ICP spectrophotometer.

Viscosities were determined with a Haake Rotovisco RV3 (Model #NR 72517, Berlin, Germany) viscometer, equipped with a NV annulus module, and a jacketed sample vessel capable of maintaining a constant measurement temperature of 20 C. Multiple measurements of the individual sample aliquots were made; due to the shear thinning nature of the systems, the average viscosity is reported for samples measured at an experimental shear rate of 242.6 s -1.

Results and Discussion

Figure III - 1 indicates the characteristic permeate flux versus average transmembrane pressure profiles of soluble and precipitated 1.2% and 0.25% whey protein solutions on the DDS Lab-20 system. A plateau indicating independence of flux with increasing pressure was not reached over the pressure range of this experiment, although the rate of increase in permeate flux as a function of applied transmembrane pressure was reduced at higher pressures. The heat precipitated whey protein solutions generally showed the same characteristic relationship between flux and applied pressure as the soluble protein solutions. However, the permeate flux of the precipitated protein solutions was noticeably lower throughout the range of experimental pressures.

Previous observations of improved UF performance with precipitated whey proteins in neutral pH conditions (Buhler et al., 1981, Hayes et al., 1974, Modler and Harwalkar, 1981, Tarnawski and Jelen, 1985) were obtained for heated whole wheys and not for more concentrated whey protein solutions. For comparison, the soluble and

precipitated whey protein solutions were diluted and processed as above. Diluting the original retentate to a level of 0.25% protein prior to ultrafiltration did not change the trends shown by the 1.2% protein solution, indicating that ultrafiltering precipitated rather than soluble whey proteins would produce no processing advantage even at relatively low concentrations.

Since all the concentrates in this experiment were processed at pH 4.0 - 4.4 which was significantly lower than the pH range of 6.0 - 6.5 used previously (Tarnawski and Jelen, 1985), the observed flux decrease caused by the heating should not have been caused by the precipitation of insoluble calcium complexes. Although a return to approximate pre-trial water flux rates could be achieved, indicating an essentially reversible flux decline, the cleaning regimen required was still excessive. This observation may implicate the precipitated whey proteins themselves in the flux decline, possibly aided by the calcium-protein and other mineral interactions upon heat aggregation (Jelen and Buchheim, 1984, Li-Chan, 1983, Sawyer , 1968, Varunsatian et al., 1983).

The permeate flux as a function of whey protein content is illustrated in Figure III - 2. It was hoped that periodic removal of the product from the system, cleaning of the membranes, and reequilibration before sampling would reduce the influence of fouling on the permeate flux/protein content relationship. As expected, the flux decreased with increasing protein content, with the precipitated protein solutions showing especially strong effects. Two separate aliquots of heat precipitated whey protein concentrate produced uncontrollably high module pressures and severe decreases in permeate flux at approximately 2:1 concentration ratio. As a result, this fraction was not included in subsequent observations. The diafiltered precipitated protein solution showed similar strong trends and thus much lower final protein concentration could be achieved.

The last data points plotted for each trial in Figure III - 2 indicate the last sampling point before the module pressure became uncontrollable. It might have been possible to concentrate the solutions to a higher degree if the module pressure was allowed to rise. It was arbitrarily decided to stop at an experimental pressure

approximately 2.5 times normal UF pressure range. The maximum protein concentrations achieved were 3.5%, 15% and 19% for diafiltered precipitated, soluble, and diafiltered soluble systems respectively. Whey protein concentrates seldom have more than 20 - 23% total solids, with the maximum total solids content achievable on the DDS Lab-20 of 35% (Rubin, 1980) at 50 °C.

The viscosities of the test solutions as a function of protein content are shown in Figure III - 3. The viscosity of the precipitated diafiltered whey protein solution increased very rapidly with protein content in comparison to the soluble systems. This obviously explains the onset of uncontrollable module pressures at viscosities of approximately 0.15 Pa·s. The viscosity limit of 0.30 Pa·s recommended for commercial DDS ultrafiltration equipment (Nielsen and Nielsen, 1985) places a severe processing constraint on the level of concentration possible with precipitated whey proteins. Heating of whey systems at pH levels close to the protein isoelectric point tends to increase viscosity (Modler and Harwalkar. 1981). A contributing factor to the poor UF performance in these trials may have been the occurrence

of dead spots on the membrane due to channeling, channel blockages, and the hydrodynamic inconsistencies of this particular apparatus (Tarnawski et al.,1984) accentuated by the high viscosity of the precipitated whey protein system.

The rejection characteristics for Ca, P and lactose in 1.25% soluble and precipitated solutions are shown in Figures III -4 and III -5. Increasing pressure appeared to increase rejection coefficients for all microsolutes observed.

Conclusions

Claims of improved ultrafiltration performance after protein precipitation could not be verified for heat precipitated acid whey protein systems processed at pH 4.0-4.4. For whey protein retentates with either 1.2% or 0.25% protein content, heat precipitating the proteins resulted in lower flux rates in the pressure range investigated. The precipitated whey protein systems exhibited an rapid drop in flux rate with increasing protein concentration when compared to similar

soluble systems. Although the flux decline for all systems could be partially compensated for by increasing module pressure, rapid increases in viscosity of the precipitated systems resulted in unacceptably high module pressures. Rejection coefficients of Ca, P, and lactose appeared to increase with increasing pressure for both soluble and precipitated systems. The observed rejections of microsolute species may indicate the potential to modify the microsolute concentrations of whey protein concentrates by manipulating the pressure and other conditions during ultrafiltration.

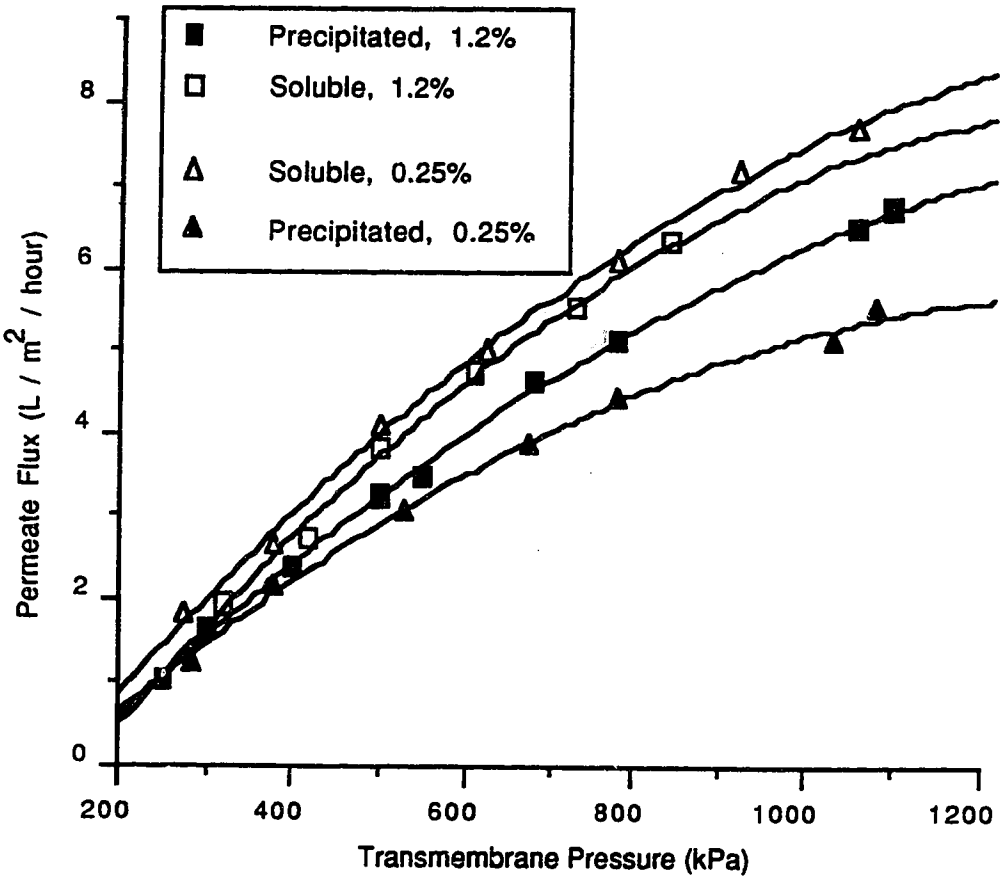

Figure III -1. Permeate Flux as a Function of Average Transmembrane Pressure

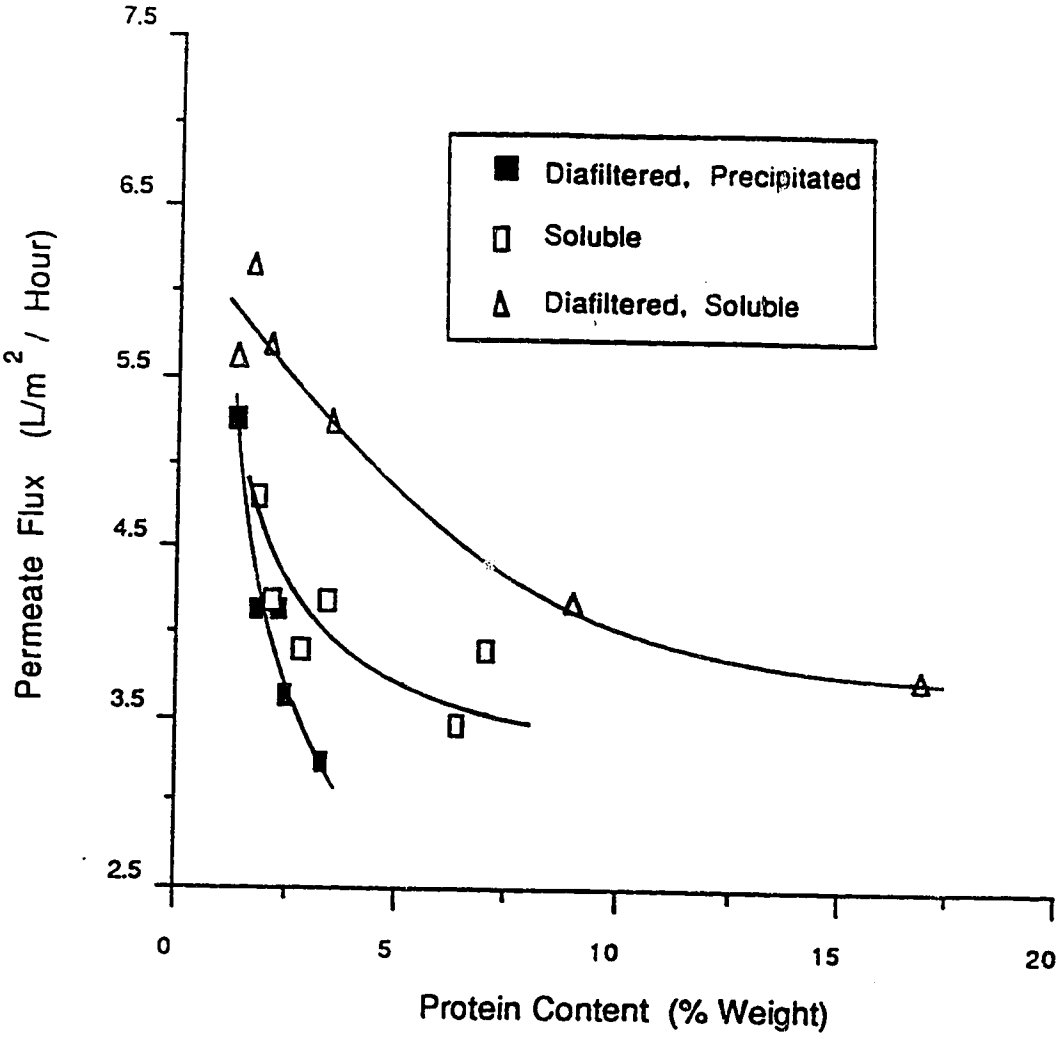

Figure III -2. Permeate Flux as a Function of Protein Content

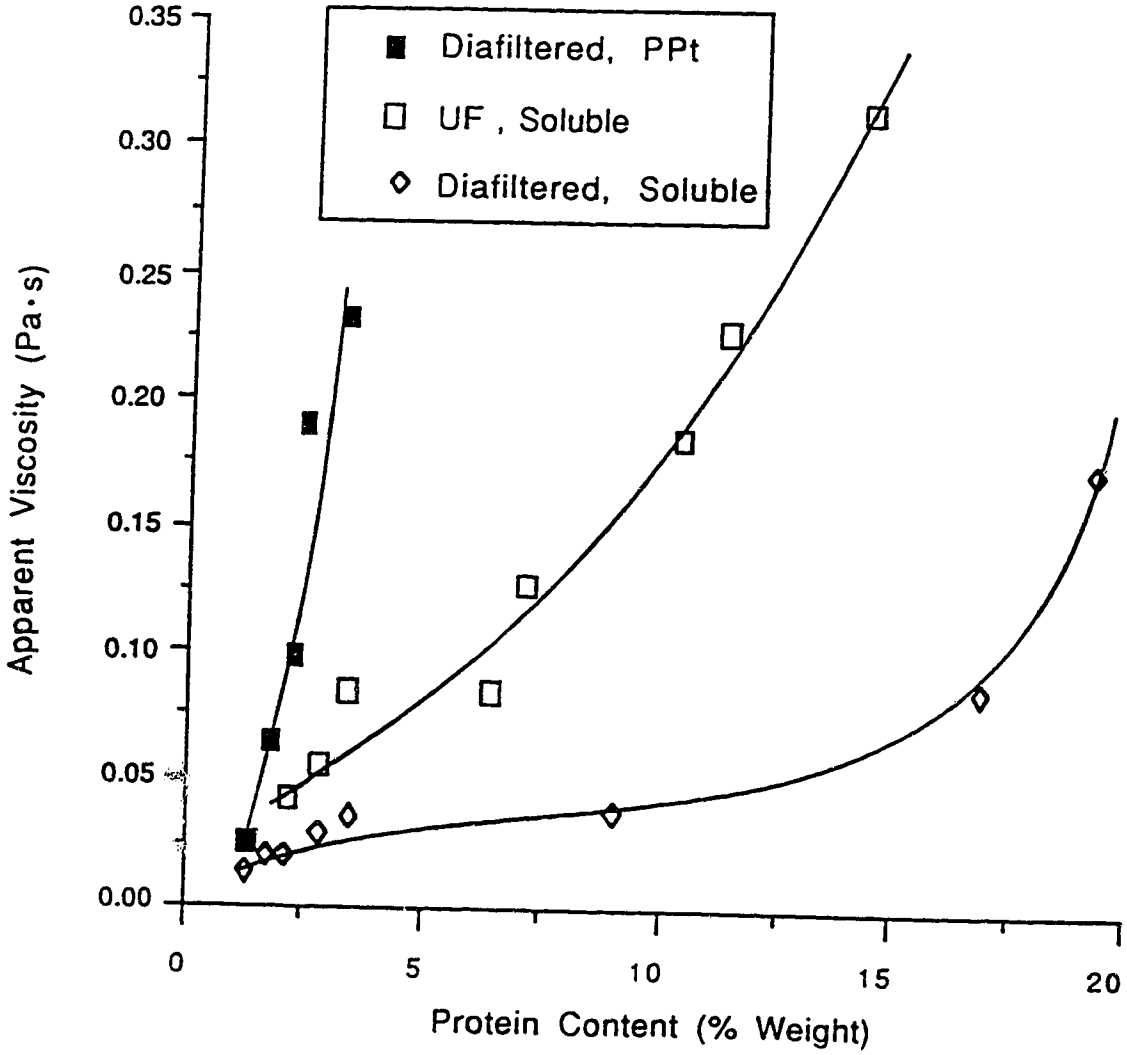

Figure III -3. Apparent Viscosity as a Function of Protein Content for Soluble and Precipitated UF Whey Protein Concentrates (shear rate= 242.6 s-1)

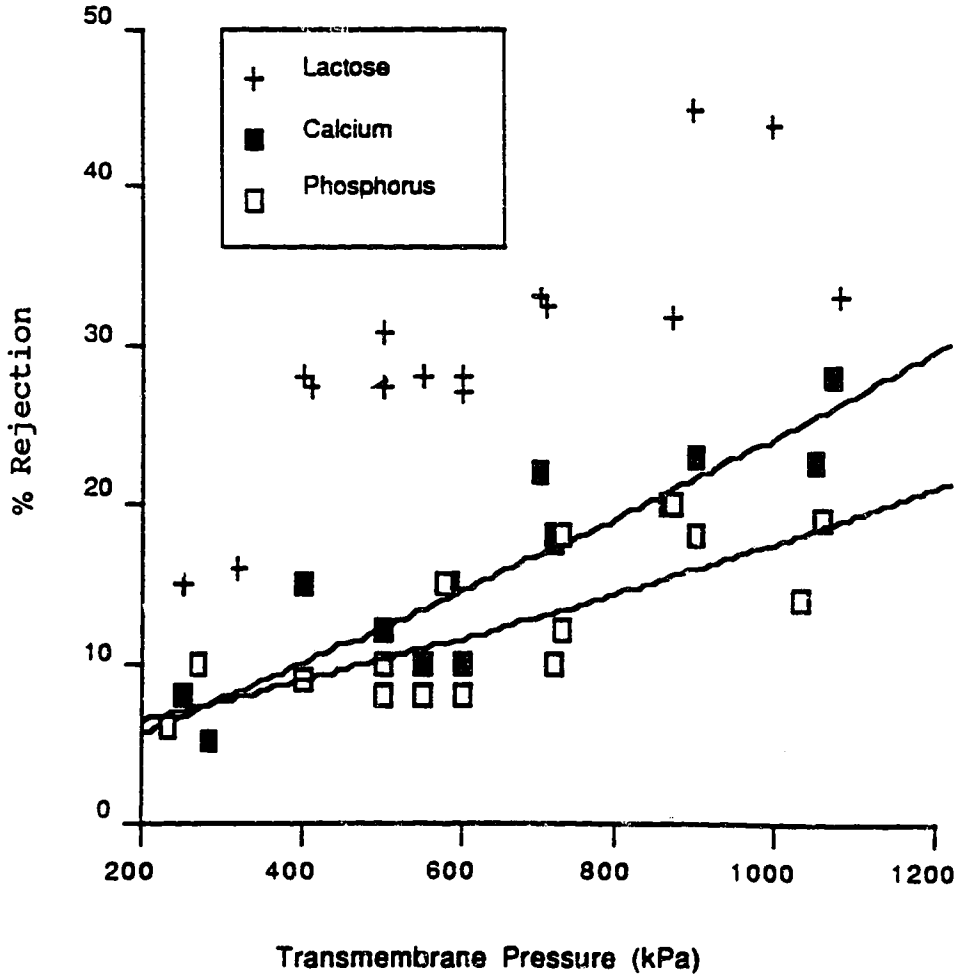

Figure III -4. % Rejection of Calcium, Lactose, and Phosphorus as a Function of Transmembrane Pressure for a Soluble 1.2% WPC Solution

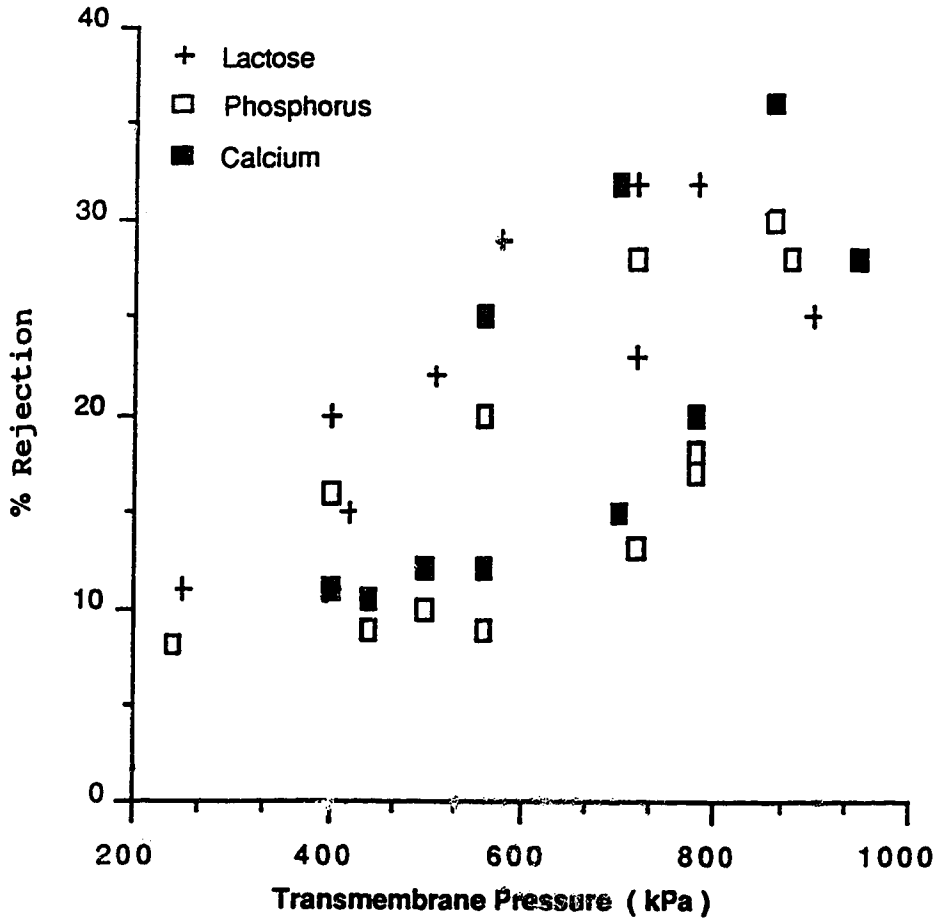

Figure III -5. % Rejection of Calcium, Lactose, and Phosphorus as a Function of Transmembrane Pressure for a Precipitated 1.2% WPC Solution

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CHAPTER IV. PARTITIONING OF MINERALS DURING ULTRAFILTRATION OF COTTAGE CHEESE WHEY

Introduction

Despite the recognized importance of minerals for whey protein concentrate functionality, as well as fouling and flux decline during ultrafiltration, little attention has been given in literature to the retentions of minerals during ultrafiltration of dairy fluids, such as whey. Typically, research reports include only the final ash or mineral constituents in UF concentrates of milk (De Boer et al., 1973, Pompei et al., 1973, Brule et al., 1974, Peri et al., 1973, Brule and Fauquant, 1981, Hansen, 1981) or whey (Matthews, 1978, Wong et al., 1978, Patocka and Jelen, 1987).

The cheese manufacturing technology -particularly the pH of dipping- has a great influence on mineral composition of wheys (Adrian and Bourlier, 1980, Van den Berg and De Vries, 1975) . High levels of milk salts may interfere with the efficiency of ultrafiltration through membrane fouling, and may produce a protein concentrate with salty taste and altered functionality.

The ultrafiltration of acid wheys is affected by high mineral contents (Patocka and Jelen, 1987). The relative ratios of individual mineral species vary predictably with the source of whey with acid wheys having a higher total calcium, phosphorus and other salt content. Acid whey can be characterized by higher calcium and phosphorus to sodium and potassium ratios in comparison to sweet whey (Wong et al., 1978). This difference is directly attributable to the increased amounts of solubilized colloidal calcium phosphate present in acid whey.

Minerals bound directly to the proteins or associated in colloidal structures would be expected to be retained at a rate proportional to the retention of the protein (Kiviniemi, 1977). A modified form of the Donnan effect has been used by Hiddink et al. (1978) to partially explain the retention of permeable ions in gouda whey during UF concentration at pH 6.6 and 3.2 due to the retention of whey proteins acting as impermeable counterions. The observed rejections of ionic species during ultrafiltration indicate that the distribution of salts between retentate and permeate is not governed entirely be the rejection characteristics of the membrane

alone (Fane et al., 1983). At present it is unknown to what extent the observed rejection of individual mineral species is affected by Donnan equilibria, intrinsic membrane rejection, concentration polarization, and the absorbed fouling layer.

Response surface methodology (RSM) is a powerful experimental approach widely exploited in industrial chemical engineering applications for determining process optima (Matthews et al., 1978, Mullen and Ennis, 1979, Biles and Swain, 1980, Fuls et al., 1987). The procedures are based on collections of central composite designs which combine experimental design, mathematical modeling, statistical inference and the most efficient use of empirical data into one unified experimental strategy. RSM is particularly advantageous to study the simultaneous effects of a multiplicity of factors (variables) on one or more responses.

The objectives of this investigation were to study the rejection patterns of calcium (Ca), sodium (Na), magnesium (Mg), potassium (K), and phosphorus (P), and to determine their relationship to the flux decline during a period of

ultrafiltration of cottage cheese whey. A five variable central composite experimental design was used to determine the effect of simultaneous variation of temperature, pressure, flowrate, pH and the addition of sequestrant (EDTA) on the observed rejection coefficients.

Materials and Methods

Fresh cottage cheese whey was obtained from a local dairy and refrigerated at 4 °C until used. The whey was not clarified, but care was taken to remove the accompanying fines by decantation. The necessary pH adjustments were made using NaOH and HCl (Fisher Scientific, New Jersey). Ethylene diamine tetraacetic acid (EDTA, BDH Chemicals, Toronto) was used as a disodium salt for the sequestrant addition.

Experimental Procedures

Ultrafiltration (UF) trials were performed on a DDS (De Danske Sukkerfabrikker, Nakskov, Denmark) Lab-20 UF unit, equipped with 2-GR-61-PP polysulphone membranes (20,000 MW cut-off) and Rannie three piston variable speed

drive pump (Model 12-18/50, Rannie Co., Copenhagen). Fluid flow rates of between 3 L/min and 8 L/min could be accurately reproduced.

The Lab-20 module included inlet and outlet pressure gauges, and a relief valve to regulate the module pressure. Each experimental trial started with thorough cleaning of the membranes as per manufacturer's instructions. Membranes were interchanged frequently to reduce possibility of long term fouling or compaction (Tarnawski and Jelen, 1986). When new membranes were installed in the UF unit, water was circulated at an operating pressure of 300 kPa for 4 hours to temper the membrane.

The procedure for each experimental trial was as indicated in Figure IV - 1. A 12 litre aliquot of whey was placed in a jacketed, temperature controlled feed tank and was allowed to equilibrate to the desired experimental trial temperature after sequestrant addition followed by pH adjustment. After 15 minutes holding at the desired temperature, the sample was recycled through the UF unit at the desired experimental pressure for 30 minutes prior

to taking initial retentate and permeate samples and initial flux measurements.

Ultrafiltration was continued under full recycle of permeate and retentate for a total of 240 minutes, after which final flux measurements, and retentate and permeate samples were taken. Samples were immediately frozen for later determination of minerals by O.S. Longman Provincial Laboratory using Inductively Coupled Plasma (ICP) Emission Spectrophotometry (Fisher Atomscan 2000 ICP spectrophotometer). Measurements of pH were made with an Orion Research (Cambridge, Mass.) model 601A Digital Ionalyzer.

Experimental Variables

A 5 variable, 5 level central composite design utilizing response surface methodology (RSM) was used to determine the interrelationship of the process parameters. A summary of the coded and real level variables and the experimental combinations is shown in Table IV - 1. The order of the experimental trials was randomized and performed in the sequence indicated. Pressure, temperature and fluid flow rate are the three operational

parameters typically controlled in UF processing, while pH was chosen as it fundamentally affects the solution characteristics (mineral solubility, ionic strength, protein charge). In cottage cheese whey where the presence of high levels of calcium affects membrane fouling, the binding of calcium in chelate complexes appears to be the best way to minimize the availability of calcium for calcium-membrane interactions resulting in flux decline (Patocka and Jelen, 1987).

Retention of an individual microsolute species was reported on a bulk concentration basis, and is expressed as:

$$\% \text{ Retention} = \left[1 - \frac{(\text{Permeate Concentration})}{(\text{Retentate Concentration})} \right] X\ 100\% \quad (1)$$

The ratio of flux measured after 240 minutes to that at 30 minutes was used as an indicator of flux decline.

Treatment of Experimental Data

Second order Taylor expansion polynomials of the form as shown in Table IV -3 were used to model the experimental responses. The coded variables were fitted to the data to obtain empirical models for each response. A response surface methodology computer package (National Food Laboratory, Dublin, Calif.) was used for designing the experiment, randomizing experimental trials and for preliminary statistical analyses of the data. Subsequent statistical analyses were performed using a University of Alberta computer package (Statpack). Significance of individual coefficients was determined by stepwise regression procedures.

Results

The poor fits ($R^2 < .60$) of empirical polynomials describing responses observed at 30 minutes operation, may have been due to the failure to rapidly achieve an

operational equilibrium. The multidimensional analysis of a tubular reverse osmosis system by Fuls et al. (1987) indicated a significant settling down period of greater than 1 hour after adjusting the parameters. Previous experience with the DDS Lab 20 UF/RO unit (Tarnawski et al., 1984) indicated an extremely complex hydrodynamic behavior that might be expected to require and extended settling down period.

The empirical coefficients of the response surfaces, fitted to the 240 minute data, are given in Table IV -2. Analysis of variance for the responses, as summarized in Table IV - 3 indicates the importance of first order, second order and interaction effects. From the square multiple correlation coefficients for the empirical models, it can be seen that the response surfaces explained a minimum of 80% of the total variation for potassium to 91% for phosphorus. All F tests for the regressions were significant at $P< 0.05$, with the exception of potassium which was significant at $P< 0.075$.

Response contours indicating the rejection of particular species as functions of the process parameters are shown in Figures IV - 2, IV - 3, IV - 4, IV - 5, and IV -6. Negative rejections of Ca, Na, P, and K were observed between retentate and permeate fractions as defined by Equation 1. Such an observation is indicative of the measured bulk concentration of a selected species being greater in the permeate than the retentate.

The axes of pH and EDTA addition used for the response contours were chosen due to their determined significance in describing the behavior of Ca and Na rejection. Reduced empirical models describing the microsolute rejections are shown in Table IV - 4. The terms of the reduced models were determined by stepwise regression and are listed in order of contribution to the overall described variation. For each of the modelled responses, the predominance of the contributions of pH and EDTA addition to the modelled response can be seen.

Response surfaces describing the rejections of microsolutes are different for each species. Divalent ions (i.e. Ca and Mg) showed more positive rejections over

the range of experimental conditions than the monovalent Na and K. This is in agreement with the observation of Hiddink et al. (1978) that divalent species are preferentially retained during whey ultrafiltration. Calcium rejections were positive except at pH values close to the isoelectric points of whey proteins (4.2 -5.2) and in the presence of more than 0.20 g/L EDTA. Magnesium exhibited a similar contour except that uniformly positive rejections were observed over the entire experimental range. For all conditions within the range of experimental parameters, the rejection of calcium and magnesium were uniformly more positive than sodium and potassium. The response contour of phosphorus closely approximates that of calcium with lower magnitudes of positive rejections. This observation is not surprising as calcium in whey is predominantly in the form of soluble calcium phosphate complexes with a part of the total calcium bound to the main protein fractions in whey, beta-lactoglobulin (Zittle et al., 1957) and alpha-lactalbumin (Hiraoka et al., 1980, Kronman et al., 1981, Bernal and Jelen, 1984).

The responses of the selected monovalent species (Na, K)

exhibited a more gradual change in observed rejection with changes in pH and sequestrant addition. Higher concentrations and less specific cation/protein interactions may have caused the smoothness of the response.

Minimum observed rejections of divalent Ca and Mg occurred approximately at the isoelectric pH of whey proteins. Away from the isoelectric point the pH change resulted in more positive rejections. Monovalent species exhibited increasing rejection with increasing pH throughout the experimental range with positive rejections observed at conditions above the isoelectric point. The different response of divalent and monovalent species to a reduction in pH below 4.5 could be attributed to the preferential interactions between the specific mineral species and the retained protein fraction. Higher charge to mass ratios of Ca and Mg compared to monovalent species (Na^+, K^+) will increase their non specific association around whey proteins. The specific binding relationships of Ca and Mg to whey proteins (Zittle et al., 1957, Arakawa and Timasheff, 1982, Murakami et al., 1982), and the association of Ca in ionic Ca-phosphate will also result

in increased associations in the retentate, and thus presumably increased rejection.

The addition of EDTA to the cottage cheese whey caused a reduction in observed rejection of all species studied with increasing sequestrant level causing progressively lower rejection. The EDTA addition was prior to pH adjustment to minimize any pH shift that might occur. Although EDTA will effectively compete for available cations with the solution proteins, it cannot be considered an impermeable counterion due to its much lower molecular weight. The influence of EDTA was most pronounced for Ca, Mg, and Na in the isoelectric range of the proteins, while potassium rejections were least sensitive probably due to high potassium concentration and lesser expected interaction with EDTA. The ability of sequestrant addition to reduce the rejection of mineral species may indicate a possibility for the use of specific chemical interactions to affect microsolute rejections.

The parameters of pressure, temperature and fluid flow rate, although contributing to the observed rejection model, did not exhibit the same dominance over the

observed responses as pH or the sequestrant. The significance of operating parameters in describing various responses indicated the contribution of operating conditions to the observed rejections. The formation of a proteinaceous dynamic membrane layer can act as a series resistance for the transport of microsolutes (Yan et al., 1979). Concentration polarization in UF resulting from the low diffusivities of macromolecules (Nakao and Kimura, 1981) can lead to significant rejections for amino acids and salts in the case of skimmilk (Kulozik, 1986) or casein hydrolysates (Walsh et al., 1987). Conditions such as increasing transmembrane pressure and decreasing flowrate would be expected to contribute to concentration polarization, and therefore to an increased microsolute rejection (Matthews et al., 1978).

The presence of a charged polyelectrolyte layer at the membrane surface has been shown to significantly affect the rejections of cations during UF (Akred et al., 1980). For the UF of salt containing gelatin solutions, negative rejections of the salt ions were observed for conditions of low solution pH, high gelatin concentration, and low transmembrane pressure. However, at operating conditions

favoring gel polarization (high transmembrane pressures), the presence of a positively charged gel layer retarded the passage of cations, leading to positive rejections of calcium ions. For the range of operating conditions studied here, the presence of a similar highly positively charged concentration polarization layer of whey proteins may explain the observed increasing rejections of cations at low solution pH.

All attempts to alter processing parameters to affect microsolute rejections must be made with due consideration of the overall process performance. Typically measured as flux and rate of flux decline, membrane performance is the most important parameter in evaluating a UF system. Plotted as a ratio of final to initial measured flux rates, the response contour for flux decline shown in Figure IV - 7 indicates that as pH increased the decrease of the 240 minute flux in relation to the initial flux was more pronounced. Ultrafiltration of acid whey solutions adjusted to pH 6 results in significant fouling (Muller and Harper, 1979). Figure IV - 7 indicates however that the addition of EDTA can preserve the flux. It may be that Ca mediates the absorption of proteins to

the membrane surface since the use of a competitive sequestrant in conjunction with lowering of pH can be used to reduce flux decline (Patocka and Jelen, 1987).

A concern with the polysulphone membrane system was the constancy of the membrane flux to changes in solution pH. The modelled responses represent the observed bulk rejections and flux decline in a situation where membrane stability is not maintained. As shown in Figure IV - 8 , the flux to transmembrane pressure profiles differ significantly for the ultrafiltration of distilled water adjusted to pH 3.35, 5.0 , and 7.8 with either hydrochloric acid or sodium hydroxide. Both polyacrylonitrile (Le and Howell, 1983) and polysulphone (Chong et al., 1985) membranes have shown water flux dependence on pH , with water flux increasing with increasing pH for the polysulphone system (Chong et al., 1985). Swelling of the polymer at lower pH may act to constrict the membrane pores resulting in a tighter membrane and reduced flux.

Conclusions

The empirical models may indicate several important trends. Over the range of experimental conditions, the models indicated more positive rejections for divalent cations (Ca, Mg) than for monovalent cations (Na, K). Calcium rejection was uniformly positive except for pH values approximating the isoelectric points of the whey proteins (pH 4.2 - 5.2) and in the presence of more than 0.20 g/L of EDTA. Increasing pH increased the observed microsolute rejections, but also increased the rate of flux decline. Increasing levels of EDTA addition decreased both the rejection of observed microsolutes and the rate of flux decline.

Process optimization in UF operations cannot be limited to optimizing permeate flux, but must also consider optimizing the process to produce the desired, high value end-products. The more positive rejections of Ca relative to Na, and the differential rejections resulting from changes in experimental parameters indicate the potential to exert some control over the mineral composition of UF retentates from milk and milk derivatives. Further

investigation of the specific mechanisms of mineral rejection will require the use of the "new generation" of more chemically and physically resistant ceramic and metal oxide UF membranes.

Table IV - 1. Five Variable , Five Level Central Composite Experimental Design With Center Points

Plan No.	Seq. No.	Variable Levels				
		(X1)	(X2)	(X3)	(X4)	(X5)
1	7	-1	-1	-1	0	1
2	5	1	-1	-1	0	-1
3	19	-1	1	-1	0	-1
4	29	1	1	-1	0	1
5	16	-1	-1	1	0	1
6	2	1	-1	1	0	1
7	11	-1	1	1	0	1
8	14	1	1	1	0	-1
9	26	-1	-1	-1	1	-1
10	18	1	-1	-1	1	1
11	32	-1	1	-1	1	1
12	10	1	1	-1	1	-1
13	6	-1	-1	1	1	1
14	30	1	-1	1	1	-1
15	12	-1	1	1	1	0
16	28	1	1	1	1	1
17	27	-2	0	0	-1	0
18	24	2	0	0	-1	0
19	3	0	-2	0	-1	0
20	8	0	2	0	-1	0
21	31	0	0	-2	0	0
22	22	0	0	2	0	0
23	13	0	0	0	-2	0
24	15	0	0	0	2	0
25	9	0	0	0	0	-2
26	17	0	0	0	0	2
27	1	0	0	0	0	0
28	4	0	0	0	0	0
29	21	0	0	0	0	0
30	20	0	0	0	0	0
31	23	0	0	0	0	0
32	25	0	0	0	0	0

Definition of Variables	(-2)	(-1)	(0)	(1)	(2)
(X1) pH of whey	3.00	4.00	5.00	6.00	7.00
(X2) Temperature of Operation (°C)	20.00	27.50	35.00	42.50	50.00
(X3) Fluid Flowrate (L / min)	2.00	3.00	4.00	5.00	6.00
(X4) Average Transmembrane Pressure (kPa)	300.0	350.0	400.0	450.0	500.0
(X5) EDTA Addition (g / L)	0.00	0.10	0.20	0.30	0.40

Table IV - 2. Taylor Expansion Coefficients (B) Describing % Retentions of Ca, Na, P, Mg, and K and Flux Decline for Ultrafiltration of Cottage Cheese Whey on a DDS Lab - 20 UF Unit

	Calcium		Sodium		Phosphorus		Magnesium		Potassium		J Final/ JInitial	
	B	(SE)	B	(SE)	B	(SE)	B	(SE)	B	(SE)	B	(SE)
pH	8.27	(2.09)	7.67	(2.58)	3.08	(1.44)	-0.40	(1.93)	7.72	(1.73)	-0.039	(0.012)
T	3.45	(2.31)	1.64	(2.86)	1.85	(1.59)	3.60	(2.14)	0.12	(1.91)	-0.063	(0.013)
F	-4.31	(2.17)	1.22	(2.68)	-3.90	(1.50)	-3.42	(2.01)	-3.00	(1.79)	-0.031	(0.012)
P	2.46	(2.17)	1.27	(2.68)	1.16	(1.50)	3.82	(2.01)	-1.39	(1.79)	0.052	(0.013)
EDTA	-4.80	(2.09)	-9.82	(2.59)	-2.89	(1.44)	-4.71	(1.94)	-1.40	(1.73)	0.039	(0.013)
pH^2	8.43	(1.72)	4.21	(2.13)	7.86	(1.19)	6.34	(1.60)	0.80	(1.42)	-0.003	(0.010)
T^2	-1.67	(1.72)	-0.45	(2.13)	-2.93	(1.19)	-2.68	(1.60)	-2.82	(1.42)	-0.006	(0.010)
F^2	0.88	(1.72)	-0.41	(2.13)	1.62	(1.19)	2.84	(1.60)	1.51	(1.42)	0.004	(0.010)
P^2	4.19	(1.72)	4.57	(2.13)	3.83	(1.19)	5.40	(1.60)	2.38	(1.42)	-0.006	(0.010)
$EDTA^2$	2.15	(1.72)	-3.83	(2.13)	0.81	(1.19)	2.44	(1.60)	-0.89	(1.42)	0.008	(0.010)
pH·T	-1.41	(3.24)	-2.53	(4.01)	-3.76	(2.24)	-3.31	(3.00)	-3.81	(2.68)	-0.076	(0.018)
pH·F	-3.19	(2.69)	2.29	(3.32)	-3.80	(1.85)	-3.69	(2.49)	-2.54	(2.22)	-0.019	(0.015)
pH·P	6.13	(2.69)	7.70	(3.32)	7.40	(1.85)	6.75	(2.49)	3.08	(2.22)	0.019	(0.015)
pH·EDTA	0.35	(2.82)	-1.64	(3.49)	1.31	(1.95)	-4.81	(2.62)	-0.54	(2.33)	0.046	(0.016)
T·F	-2.53	(3.06)	-5.00	(3.78)	1.33	(2.11)	0.61	(2.84)	0.44	(2.53)	0.048	(0.017)
T·P	-2.59	(3.06)	0.25	(3.78)	-6.13	(2.11)	-4.02	(2.84)	0.49	(2.53)	-0.020	(0.017)
T·EDTA	-2.63	(3.24)	6.48	(4.01)	0.06	(2.23)	4.98	(3.00)	0.25	(2.68)	0.006	(0.018)
F·P	0.46	(2.82)	1.30	(3.50)	-2.76	(1.95)	-1.68	(2.61)	-1.28	(2.33)	-0.013	(0.016)
F·EDTA	-0.69	(2.69)	4.12	(3.32)	1.77	(1.85)	2.01	(2.49)	0.83	(2.22)	-0.009	(0.015)
P·EDTA	5.53	(2.68)	-0.49	(3.32)	4.57	(1.85)	-0.25	(2.49)	2.27	(2.22)	0.024	(0.015)
BO	1.10		-11.6		-1.94		3.97		-11.05		0.7	

SE = Standard Error

Table IV -3. Summary of Analysi[illegible]f 2nd Order Taylor Expansion Polynomials Describing Microsc[illegible]nd Flux Decline as a Function of Transformed Variab[illegible]

$$Y = B0 + [B1(pH) + [illegible](FF) + B4(P) + B5(EDTA)]$$
$$+ [B6(pH^2) + [illegible](FF^2) + B9(P^2) + B10(EDTA^2)]$$
$$+ [B11(pH \cdot T) + [illegible]13(pH \cdot P) + B14(pH \cdot EDTA) + B15(T \cdot FF)$$
$$+ B16(T \cdot P) + [illegible](T \cdot EDTA) + B18(FF \cdot P) + B19(FF \cdot EDTA) + B20(P \cdot EDTA)]$$

	DF	RET. Ca	RET. Na	RET. P	RET. Mg	RET. K	JFINAL/ JINITIAL
		----------	----------	% TOTAL	VARIANCE	----------	----------
First Order	5	39.37**	41.00**	15.60*	18.90*	50.05**	50.26**
Second Order	5	35.16**	18.74	45.08**	34.95**	17.50	3.27
Interaction	10	13.73	24.24	30.62*	34.91*	13.04	30.81
Lack of Fit	6	9.25	11.89	6.08	8.84	9.98	13.54
Residual Error [1]	5	2.49	4.13	2.62	2.40	9.44	2.12
Total	31						
R^2=		0.939	0.916	0.956	0.942	0.898	0.918
R^2=		0.883	0.840	0.913	0.887	0.806	0.843

1 Significance of regression contribution evaluated with pooled residual MS.
** Highly significant ($P < 0.01$)
* Significant ($P < 0.05$)