PROCESS ANALYSES AND CHARACTERIZATION OF LIQUID VIRGIN WHEY PROTEIN ISOLATE

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In the food industry, native whey proteins (WP) are desirable because of their high nutritional quality and excellent functional properties. In this study, *virgin whey* (VW) was harvested as permeate in the microfiltration of slightly acidified skim milk prior to cheesemaking. Free of cheesemaking remnants, bacteria and spores, VW did not require pretreatment before concentration by ultrafiltration (UF). Not exposed to extreme physicochemical conditions of cheesemaking, the WP in VW retained their native conformation. Therefore, both protein-protein and membrane-protein interactions were minimal during UF, enabling VW concentration by UF alone at reasonable flux. This allowed the production of liquid virgin whey protein isolate (LVWPI) containing up to 26% total solids, about 91% of which was WP.

The LVWPI is a novel ingredient rich in native WP and of low mineral content. It showed unique physicochemical properties and functional behavior not observed in commercial WP products. It exhibited low viscosity and thermal stability against rapid aggregation that led to controlled heat-induced aggregation and gelation suitable for fine-tuned food texturization. It was produced by concentrating VW at 45 °C using pilot-scale two-stage UF system with polysulfone membranes (10-kDa molecular weight cut-off). VW was first concentrated ~13x in a spiral wound module (SWM), and then diafiltered to achieve ~99% lactose removal before further concentrating ~5x in a hollow fiber module. SWM flux data showed as much as six

times increase compared to those observed in the UF of cheese whey, resulting in lower process energy requirements.

To understand the unique UF fouling behavior of VW, a two-parameter flux model was derived. One parameter, expressed as the ratio of feed stream (F) to membrane area (A), quantified membrane-protein interactions that give rise to initial flux decline. Another is the long-term fouling parameter, m, which indicated protein-protein interactions. Results showed that m was constant, regardless of F value, due to VW's consistent composition. However, initial flux decline depended on F/A. The model proved to be a practical design equation for optimum F/A in UF systems.

Finally, a technology transfer model was designed wherein a developing country benefits from the LVWPI technology developed in this study.

BIOGRAPHICAL SKETCH

Philipina A. Marcelo was born on July 3, 1968 in Manaoag, Pangasinan, a province in the northern island of Luzon in the Philippines. She obtained Bachelor's Degree in Chemical Engineering from the Pontifical and Royal University of Santo Tomas (UST) in Manila, Philippines in 1989. She joined the UST Department of Chemical Engineering as teaching assistant and laboratory instructor, right after graduation. A year later, she passed the Chemical Engineering Licensure Examination given by the Philippine Professional Regulation Commission. In 1991, while teaching full-time at UST, she joined the Graduate Program of the University of the Philippines in Diliman, Quezon City as a part-time student, and graduated with a Master of Science in Chemical Engineering degree in 1994. She then started a tenure track appointment at UST where she became the Chem. E. Department Laboratory Supervisor. In 1995, she went to the Chem. E. Department of Ohio State University as a visiting scholar, under the auspices of the Philippine Department of Science and Technology. Upon her return to UST in 1996, she also joined the UST Graduate School, teaching in the Master of Engineering Program (MEP). In 1999, she went to the Food Engineering Department of the Lund University in Sweden as a visiting scholar and research fellow. She then became the MEP coordinator in UST upon her return in 2000. In August 2002, she joined the Ph.D. Program in Food Science at Cornell University as a Fulbright scholar while on study leave from UST. Aside from pursuing a career in the Academia and volunteer works in feeding the hungry and providing education to underprivileged children, Philipina is also a creative writing and photography enthusiast. She hopes to find the time to learn SCUBA diving, fly a little aeroplane, and to have a one-woman photography show at some point in her life.

To my beloved late mother, whose dedication as a mother, wife and school teacher has been a constant source of inspiration.

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To my siblings, just because we are all in this together.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

CF	concentration factor
CLSM	confocal laser scanning microscope
CU	Cornell University
DA	Department of Agriculture
DF	diafiltration
DOST	Department of Science and Technology
DSC	differential scanning calorimeter
FNRI	Food and Nutrition Research Institute
HFM	hollow fiber module
IE WPI	commercial whey protein isolate manufactured by ion-exchange
LVWPI	liquid virgin whey protein isolate
MCF	mass concentration factor
MF	microfiltration
MF-UF WPI	commercial whey protein isolate manufactured by microfiltration and ultrafiltration
MWCO	molecular weight cut-off
NDA	National Dairy Authority
NPN	non-protein nitrogen
PAGE	polyacrylamide gel electrophoresis
PEM	protein energy malnutrition
PSf	polysulfone
SDS	sodium dodecyl sulfate
SMC	San Miguel Corporation
SWM	spiral wound module

TMP	transmembrane pressure
TS	total solids
UF	ultrafiltration
UST	University of Santo Tomas
VW	virgin whey
WP	whey proteins
WPC	whey protein concentrate
WPI	whey protein isolate

LIST OF SYMBOLS

A	membrane area, m^2
b	long-term fouling parameter in the Merin-Cheryan flux equation = 0.12 for VW
C	concentration of true proteins in the retentate
C_p	concentration of true proteins in the permeate
C_o	concentration of true proteins in the feed
d_h	flow hydraulic diameter at the retentate side, m
E_A	activation energy of flow
E_{pp}	pump energy requirement, J kg^{-1} permeate
E_{total}	total pumping energy requirement, J
f'	Fanning-Darcy friction factor
F	amount of feed, kg
g_c	flow constant, kg m N^{-1} s^{-2}
G'	storage modulus, Pa
J	permeate flux, kg h^{-1} m^{-2}
J_o	initial permeate flux, kg h^{-1} m^{-2}
k	Merin-Cheryan equation constant
L	length of membrane module, m
L, *a*, *b*	Hunter color space coordinates (*L* = lightness, *a* = red to green, *b* = yellow to blue)
m	long-term fouling parameter in the new flux model developed = 0.89 for VW
P_i	entrance pressure, Pa
P_o	exit pressure, Pa

R	membrane rejection coefficient
t	processing time, min
T	temperature, °C
V	amount of permeate, kg
V_R	amount of retentate, kg
W_s	shaft work, J kg^{-1} of feed
ΔE	Hunter's total color difference
ΔP	pressure drop, Pa
α	virgin whey protein concentration parameter
β	liquid virgin whey protein viscosity parameter
η	apparent viscosity, mPa-s
$\bar{v}$	average crossflow velocity, m s^{-1}
τ_w	shear stress at the membrane wall, retentate side, Pa
υ	specific volume of feed, m^3 kg^{-1}
ΣF	friction losses, J kg^{-1} feed

CHAPTER 1

Physicochemical properties of liquid virgin whey protein isolate

1.1. ABSTRACT

Liquid virgin whey protein isolate (LVWPI) was produced by concentrating and fractionationating *virgin whey*, the permeate from microfiltration of acidified (pH 6) skim milk before cheesemaking. The virgin whey was subjected to a two-stage ultrafiltration system, which consisted of spiral wound and hollow fiber polysulfone membrane (10 kDa molecular weight cut-off) modules. The LVWPI contained 26.1% (w/w) total solids, about 91% of which was whey proteins. Density and viscosity at 20 ºC were 1.11 $g \cdot mL^{-1}$ and 11.65 mPa·s, respectively. The pH was constant for 38 days at 4 °C. Apparent viscosity at 122.3 s^{-1} shear rate and activation energy of flow were lower than those of whey protein isolate (WPI) and concentrate (WPC-80) solutions at 10 to 50 °C and 5 to 25% (w/w) whey protein concentrations. The LVWPI apparent viscosity after heating became identical to unheated WPI solution. The results of the study indicate that LVWPI was richer in native WP than commercial products and may serve as an excellent source of easy-to-use and nutritionally superior whey proteins.

1.2. INTRODUCTION

High nutritional quality, potent biological activity and unique functional properties are the foremost attributes of whey proteins (WP) that help sustain interest in their utilization, not only in the food industry but also in allied areas such as the pharmaceutical and biomedical fields (de Wit, Klarenbeek, & Hontelez-Backx, 1983; Bounous & Gold, 1991; McIntosh, *et al*., 1998; Tomé, 2001; de la Fuente, Singh, & Hemar, 2002b; Walzem, Dillard, & German, 2002; Ha & Zemel, 2003; Xiao, Ould Oleya, & Gunasekaran, 2003; Etzel, 2004; Bhattacharjee, Bhattacharjee, & Datta, 2006).

WP are most commonly utilized as spray dried whey protein concentrate (WPC) with 35-80% protein content or as whey protein isolate (WPI) with 80-95% protein content, which are produced from "classic" cheese whey (Brans, Schröen, van der Sman, & Boom, 2004). The loss of native conformation during cheesemaking and subsequent processing alter their functionality and reduce their biological activity (Patel, Kilara, Huffman, Hewitt, & Houlihan, 1990; Kilara & Mangino, 1991; Bounous *et al*., 1991; de Wit, 1998; Vardhanabhuti & Foegeding, 1999; de la Fuente, Hemar, Tamehana, Munro, & Singh, 2002a). Thus, practicing the appropriate conditions to achieve desired functionality in the commercial WP products remains a challenge to date (Etzel, 2004; Onwulata, Konstance, & Tomasula, 2004; Fachin & Viotto, 2005). Although proteins could assume many three-dimensional shapes, only the "native conformation" may be biologically significant (Dybing & Smith, 1991). For these reasons, interesting findings on the benefits of WP continue to multiply in the literature, and the interest in native WP of undiminished biological activity and uniform functionality continues to grow (Bhattacharjee *et al*., 2006).

The liquid virgin whey protein isolate (LVWPI), produced in our laboratory using a combination of membrane separation techniques, is an ingredient rich in native

WP that has the potential of fully exploiting the inherent nutritional, biological and functional attributes of WP. "Virgin whey" (VW) is obtained as permeate from microfiltration (MF) of slightly acidified skim milk prior to cheesemaking. It is free of fat, casein, spores, bacteria and cheesemaking foulants (Brandsma & Rizvi, 2001; Ardisson-Korat & Rizvi, 2004) and, therefore, does not require any pretreatment prior to concentration and fractionation by ultrafiltration (UF). Unlike "classic" cheese whey, VW is compositionally invariant with the type of cheese subsequently made from the MF retentate. The native conformation of the WP in the LVWPI is likely to be maintained because these proteins were not exposed to extreme physicochemical conditions of cheesemaking and because only membrane technology, which is a "gentle technology", was used in the recovery and concentration processes with no subsequent spray drying.

Although the numerous benefits of native WP are amply presented in the literature, there has been limited effort in producing and characterizing a product that contains high-purity, native WP. In this work, our objective was to produce LVWPI using a combination of membrane technologies and to characterize its physicochemical properties.

1.3. MATERIALS AND METHODS

1.3.1. Materials

High-temperature, short-time (HTST) pasteurized skim milk was obtained from the Cornell Dairy Plant and held overnight at 4 °C. The commercial WPC-80 used had an average % (w/w) composition of 79.9% protein, 6.4% fat, 2.6% ash, 6.3% lactose and 4.9% moisture while the commercial WPI used contained 90.1% protein, 0.8% fat, 2.8% ash, 1.5% lactose and 4.7% moisture as per chemical analyses made in

our laboratory. Both products were manufactured from sweet whey, concentrated by UF and then spray dried. Other chemicals used were analytical grade.

1.3.2. Production of LVWPI

The production schematic of LVWPI is shown in Figure 1.1 and is described in detail below.

Stage 1: Recovery of virgin whey by microfiltration

The MF system was a megaloop, configured for operating at a uniform transmembrane pressure (TMP) and consisted of 38 Membralox® ceramic membrane elements (Pall Corporation, Deland, FL, USA) with 0.1-μm nominal pore diameter. The elements were 1.02 m long providing an effective filtration area of 9.2 m^2. The filtration process was started by circulating 130 kg of reverse osmosis (RO)-purified water, the volume of which corresponded to the dead volume of the MF system. The water was circulated until a steady-state operation at 50 °C and UTMP of 101 kPa was attained as described by Ardisson-Korat *et al.* (2004). At this point, 1047 kg skim milk, which was gradually acidified to pH 6.0 using glucono-δ-lactone prior to MF as detailed by Brandsma and Rizvi (1999) was fed to the megaloop. The RO water diluted the skim milk, giving a dilute permeate at initial flux of about 115 $kg \cdot h^{-1} \cdot m^{-2}$. The retentate stream was then concentrated to a mass concentration factor (MCF) of 8. About 1025 kg of VW was collected, held with constant stirring at 45 °C and subsequently used as feed to the next stage.

Stage 2: Initial ultrafiltration and diafiltration of virgin whey

The initial UF concentration was carried out using S4-HFK-131-VSV polysulfone (PSf) membrane in spiral wound (SW) configuration from Koch Membrane Systems, Inc. (Wilmington, MA, USA) with a molecular weight cut-off

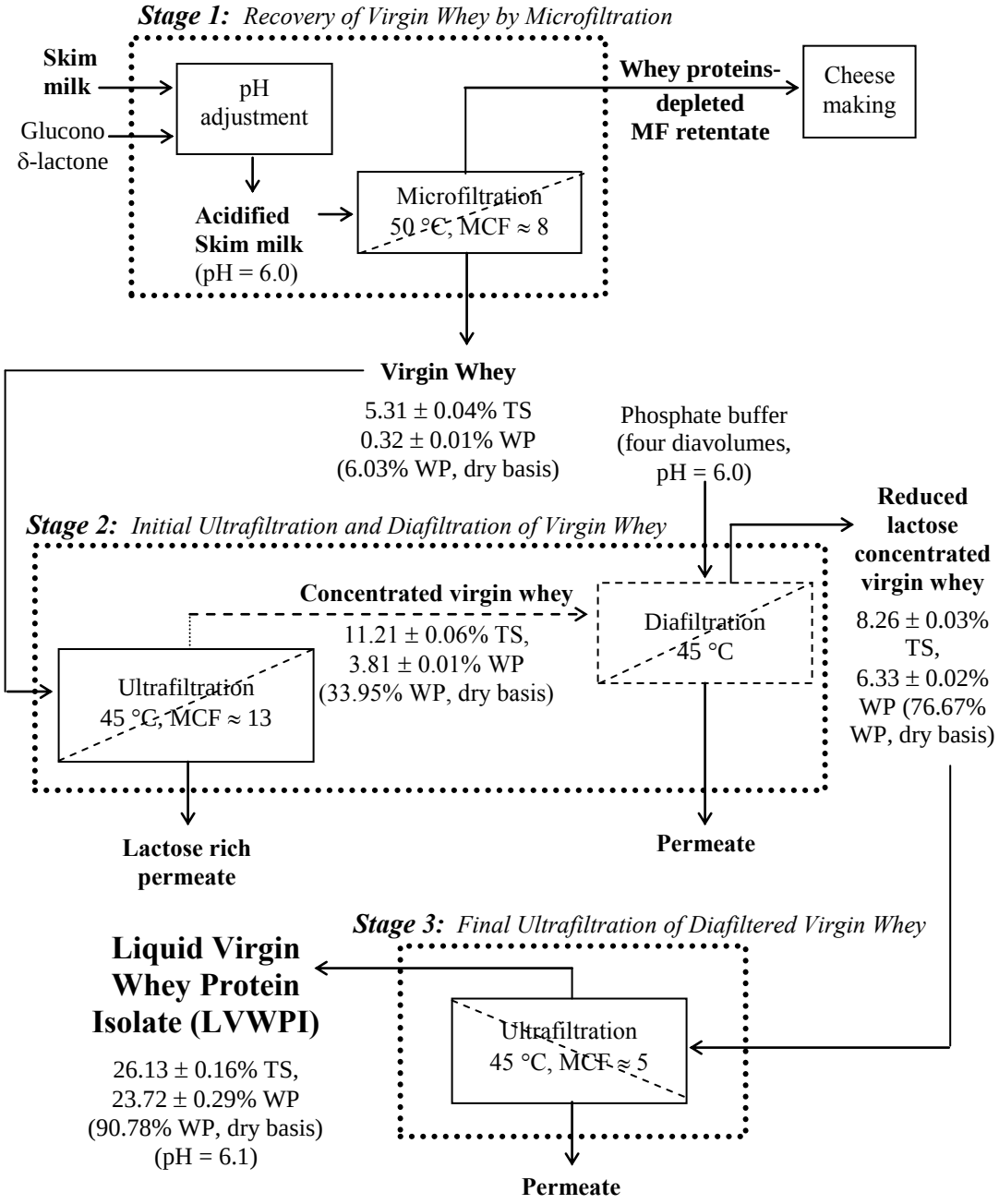

Figure 1.1. Production schematic for liquid virgin whey protein isolate (LVWPI): ***Stage 1 -*** Recovery of virgin whey (VW) by microfiltration using tubular ceramic membranes of 0.1-μm pore size and 9.1 m^2 filtration area; ***Stage 2 -*** Initial ultrafiltration and diafiltration of VW using PSf membrane area in spiral wound configuration with MWCO of 10 kDa and 5.9 m^2 filtration; ***Stage 3 -*** Final ultrafiltration of VW to produce the LVWPI using PSf membrane (10 kDa MWCO) in hollow fiber configuration with 2.9 m^2 filtration area .

(MWCO) and a total membrane area of 10 kDa and 5.9 m^2, respectively. It was operated at constant temperature of 45 °C at a tangential velocity of 0.5 $m \cdot s^{-1}$. The retentate side inlet and outlet pressures were maintained at 475 and 200 kPa, respectively, giving an average pressure differential (ΔP) along the module length of 275 kPa and an average TMP of 338 kPa. Filtration was continued until the MCF was about 13 and the WP concentration was approximately 3%.

Using the same SW system, the retentate was then diafiltered using four diafiltration (DF) volumes of phosphate buffer, which is known to maintain pH between about 5.9 and 7.9 (Nelson & Cox, 2000). The buffer system was prepared from 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 in 7:1 volumetric ratio and then diluted by 67% (v/v) RO water (Segel, 1976). This maintained the pH of the retentate at 6.0. The number of minimum DF volumes to give the minimum DF time and maximum flux for a final product of at least 90% WP purity (dry basis) was calculated by the method detailed by Glover (1985). Results from previous pilot-scale test runs were used in the calculations.

Stage 3: Final ultrafiltration of reduced lactose virgin whey

Immediately after DF, about 33 kg of the SW retentate was fed to a PSf hollow fiber (HF) membrane module, which consisted of 3" HF-25-43-PM10 from Koch Membrane Systems, Inc. (Wilmington, MA, USA) with a MWCO of 10 kDa and a total filtration area of 2.9 m^2. The operation was carried out at 45 °C and an average crossflow velocity of 2.0 $m \cdot s^{-1}$. The permeate side was open to atmosphere while the inlet and outlet pressures on the retentate side were maintained at around 300 and 170 kPa, respectively. This maintained the ΔP at 130 kPa and the average TMP at 235 kPa. The operation continued until the MCF was about 5, giving a total MCF of about 65.

The flow rates of permeate streams from the SW and HF modules were measured at 10-minute intervals. The pH and temperature of the retentate were also recorded at the same time intervals. Samples of the UF retentate and permeate at different concentration levels before and after DF were collected, immediately cooled, stored at 4 °C and their viscosity, density, color and total solids (TS) content determined within two days after production. Some samples were frozen in liquid nitrogen after the UF process and stored at -40 °C until chemical analyses.

To simulate the behavior of LVWPI at different WP concentrations, three kilograms of fresh LVWPI, in three one-kilogram batches, were freeze-dried using the Labconco bench-top freeze-drier (Kansas City, MO, USA). Freeze-drying was employed because this drying method has minimal adverse effects on the native conformation of proteins, therefore the WP in the rehydrated powder will behave in the same manner in solution as the WP in fresh LVWPI. The freeze-dried LVWPI was ground to approximately 100 μm particle size and analyzed for its chemical composition. The freeze-dried LVWPI was used to compare the flow properties of LVWPI at different protein concentrations and temperatures with those of solutions of commercial WPI and WPC-80.

1.3.3. Compositional analyses

The LVWPI composition was determined following the AOAC (2000) protocol unless otherwise specified. The %TS was determined by drying in an oven at 100 °C for four hours (AOAC, 2000; 33.2.44, 990.20). Total nitrogen was determined by Kjeldahl method (AOAC, 2000; 33.2.11, 991.20) and the true protein was obtained after correction for non-protein nitrogen (NPN) (AOAC, 2000; 33.2.12, 991.21) using a protein conversion factor of 6.38. The sample size was adjusted so as to contain similar absolute amount of protein as milk in the recommended amount in the

procedure, taking into account the concentration factors at different stages in the process. The true protein fraction was taken as equal to whey rotein fraction. Fat was analyzed using Mojonnier extraction procedure (AOAC, 2000; 33.2.26, 989.05). The ash content was determined by ashing the samples at 550 °C in an electric muffle furnace. Lactose was calculated by difference between the TS and other solid components. All determinations were done in quadruplicates. The same analyses were done on freeze-dried LVWPI and the commercial products.

1.3.4. Property characterization

pH

The pH of fresh LVWPI was measured at 20 ± 1 °C. The pH was then monitored until it started to drop from its initial value. Fresh LVWPI (500 mL) was cooled in a sterile container immediately after production and stored in a refrigerated room at 4 ± 0.5 °C. Approximately 10-mL sample of the refrigerated LVWPI was carefully poured in a polypropylene vial 24 hours after storage and its pH measured at 20 ± 1 °C immediately after adjusting its temperature in a water bath. This step was repeated at 24-hour intervals.

Color

The Macbeth® Color-Eye® spectrophotometer, model 2020 (Kollmorgen Instruments Corp., Newburgh, NY, USA) with Optiview® software was used. The Hunter values, *L, a* and *b*, of fresh LVWPI and those of WPI and WPC-80 solutions of the same protein concentration at 20 °C and pH of 6.1 were computed with the diffuse reflectance data. The total color difference, ΔE, between LVWPI and the solutions prepared from commercial products of the same WP concentration were then calculated.

Density

The density of fresh LVWPI was measured at 10, 20, 30, 40 and 50 °C using the Fisher Scientific (Suwanee, GA, USA) constant-volume pycnometer, which was initially calibrated with water.

Viscosity

The viscosity of fresh LVWPI was determined using Brookfield DV-II Viscometer (Middleboro, MA, USA) equipped with UL adapter and Wingather software for flow analysis. The viscosity was measured at constant shear rate of 122.3 s^{-1}. Only data at 10% torque and greater were considered to ensure that the readings were within the instrument's calibration range.

To determine the viscosity profile of LVWPI at different protein concentrations and to compare with those of commercial WPC-80 and WPI, appropriate amounts of WPC-80, WPI and freeze-dried LVWPI powders were dissolved in 100 mL of deionized water to make 5, 8, 10, 12, 15, 20 and 25% (w/w) protein solutions as described by Morr *et al*. (1985). The dispersions were stirred for a total of 90 minutes at room temperature and allowed to equilibrate overnight at 4 °C. The pH was then adjusted to 6.0 ± 0.1 using 0.1M NaOH or HCl as needed. Viscosities of the resulting solutions were measured at 10, 20, 30, 40 and 50 °C in triplicate. Size 75 Cannon-Fenske routine viscometer, previously calibrated with water, was used to analyze samples of viscosities less than 2 mPa·s, otherwise the Brookfield DV-II Viscometer (Middleboro, MA, USA) was used.

Activation energy of flow

To quantify the sensitivity of LVWPI viscosity to heat, the activation energy of flow of the freeze-dried LVWPI solutions was determined using the viscosity data

obtained as described above. An Arrhenius type relation was utilized as is commonly done to describe the temperature dependence of rheological parameters (Herceg & Lelas, 2005): $\eta = \eta_0 e^{\frac{E_A}{RT}}$, where: E_A = activation energy ($J \cdot mol^{-1} \cdot K^{-1}$), η_0 = pre-exponential factor (mPa·s), and R = universal gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$). The same analysis was made on WPI and WPC-80 solutions.

Heat treatment

WP solutions (20 mL) at pH 6.0 ± 0.1 with the same protein concentration as the fresh LVWPI were heated at 65 °C for 2 minutes to induce structural unfolding of the proteins without massive aggregation (Dybing *et al*., 1991; Fachin *et al*., 2005). The heating rate from room temperature to 65 °C was about 5 $°C \cdot min^{-1}$. Fractional denaturation was ascertained by measuring the reduction of the enthalpy of denaturation of the samples after heat treatment using a differential scanning calorimeter (DSC), DSC Q10 (TA Instruments, New Castle, DE, USA), between 20 and 120 °C at a scanning rate of 2 $°C \cdot min^{-1}$. The heated samples were immediately cooled to 4 °C and kept overnight prior to viscosity and flow behavior measurements. The same treatment was followed with WPI and WPC-80 solutions.

1.3.5 Statistical analyses and mathematical modeling

All statistical analyses were done using MINITAB® release 14 statistical software (State College, PA, USA). Mathematical modeling on the measured viscosities of LVWPI, where apparent viscosity was expressed as a function of both concentration and temperature, were done using MathWorks MATLAB® 7.0.4 software (Natick, MA, USA). The Arrhenius type relation was extended into a two-parameter model that considers the effects of protein concentration, temperature and other factors, such as protein-protein interactions. To determine the values of the

parameters, a nestled iterative computer program was written in Matlab code using the % relative error test between the predicted and measured viscosities at a tolerance level of 10% or less to terminate iterations. The code was used to derive the viscosity model for LVWPI.

1.4. RESULTS AND DISCUSSION

1.4.1. Production of LVWPI

The VW, which was at pH 6, was a low-viscosity, low-solute concentration feed stream, thus a combination of high TMP and low flow rate was used in the initial UF to attain reasonable permeate flux (Aimar, Taddei, Lafaille, & Sanchez, 1988; Marshall, Munro, & Trägårdh, 1993; Brans *et al*., 2004) in a cost-effective way using PSf membrane in SW configuration (Cheryan & Kuo, 1984; Brans *et al*., 2004). Starting from a clean water flux of 62.2 $kg \cdot hr^{-1} \cdot m^{-2}$, the average permeate mass flux in the SW module was 42.9 $kg \cdot hr^{-1} \cdot m^{-2}$ during the first 10 minutes of operation. Over the next 30 minutes, the flux declined exponentially with time to 34.6 $kg \cdot hr^{-1} \cdot m^{-2}$, and plateaued at about 30 $kg \cdot hr^{-1} \cdot m^{-2}$ until an MCF of about 8. The flux then declined to an average of 18 $kg \cdot hr^{-1} \cdot m^{-2}$ as the MCF reached 13 and the retentate TS was about 11% (w/w), corresponding to about 3% (w/w) WP. Above this WP concentration, Kuo and Cheryan (1983), and Nilsson (1988) found considerable drop in permeate flux. Therefore, DF was commenced at this point. At the end of DF, about 95% removal of lactose was achieved and the SW retentate TS was about 8% (w/w) with about 6% (w/w) WP (~76% WP, dry basis). During DF, the viscosity of the retentate at 20 °C remained approximately constant at 2.1 mPa·s even as significant changes in its composition occurred.

In the final UF, the HF was utilized because such configuration is known to promote high shear for the same pressure drop as in other membrane configurations

and is, therefore, capable of maintaining reasonable flux even at high feed concentration (Kuo *et al*., 1983). In this stage, an exponential flux decline with time was observed as the retentate viscosity, measured at 20 °C, increased considerably to about 11.7 mPa·s, which is almost six times its viscosity after DF. The highest and the average permeate fluxes were 22 $kg \cdot hr^{-1} \cdot m^{-2}$ and 6 $kg \cdot hr^{-1} \cdot m^{-2}$, respectively, higher than those observed by Cheryan *et al*. (1984), and Castro and Gerla (2005) in the UF of cheese whey using PSf membrane also in HF configuration.

1.4.2. Composition and physical characteristics

As shown in Table 1.1, the diluted VW harvested from skim milk as MF permeate had a pH of 6.0 and contained 5.31% TS (w/w). Its total protein content was 0.49% (w/w), or 0.32% (w/w) WP. Its density and viscosity at 20 °C were 1.04 $g \cdot cm^{-3}$ and 1.6 mPa·s, respectively. The final LVWPI had a pH of 6.1, which remained constant for 38 days at 4 °C storage temperature. It contained 26.13 % (w/w) TS, which is about 91% (w/w) WP on dry basis (Table 1.1). The fraction of components in LVWPI was generally comparable with that of WPI, except that fat was not detected in LVWPI. The freeze-dried LVWPI contained 0.7% (w/w) moisture but there was no significant difference between its dry basis composition and that of LVWPI ($p \leq 0.05$).

LVWPI was a light brown liquid at 20 °C. In colorimetric analysis, a sample is generally considered to match another if its ΔE value, computed using the L, a and b values of the latter as a reference, is equal or less than 1.0 (Francis & Clydesdale, 1975). In many food applications, the colorimetric analyses are coupled with sensory analyses to determine color difference threshold, which may give ΔE values slightly greater than 1.0 (Buffa, Trujillo, Pavia, & Guamis, 2001; Rohm & Jaros, 1996). Considering these, the LVWPI is different from both commercial WPI and WPC-80 as

Table 1.1. Gross composition of virgin whey (VW), liquid virgin whey protein isolate (LVWPI), freeze-dried LVWPI, spray dried LVWPI, and commercial WPI and WPC-80.

Component	% Composition (w/w)					
	This study				Commercial products	
	VW	LVWPI	Freeze-dried LVWPI	Spray dried LVWPI	WPI	WPC-80
Total solids[a]	5.31 ± 0.04	26.13 ± 0.16	99.29 ± 0.01	95.22 ± 0.07	95.30 ± 0.20	95.10 ± 0.26
True protein[a,b]	6.03 ± 0.05	90.78 ± 0.70	90.10 ± 0.19	89.35 ± 0.25	89.66 ± 0.13	81.94 ± 0.34
NPN[a,b,c]	3.28 ± 0.01	4.92 ± 0.13	5.13 ± 0.11	5.19 ± 0.02	4.83 ± 0.13	2.08 ± 0.10
Fat[a,b]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.79 ± 0.04	6.68 ± 0.12
Lactose[a,b,d]	83.72 ± 0.18	1.60 ± 0.63	1.69 ± 0.45	2.47 ± 0.20	1.60 ± 0.09	6.58 ± 0.16
Ash[a,b]	6.97 ± 0.07	2.72 ± 0.06	3.08 ± 0.02	2.99 ± 0.03	3.12 ± 0.09	2.73 ± 0.08
Water[a]	94.69 ± 0.04	73.87 ± 0.16	0.71 ± 0.01	4.78 ± 0.07	4.76 ± 0.14	4.90 ± 0.08

[a] Mean of quadruplicates ± standard deviation.
[b] Dry basis.
[c] Non-protein nitrogen.
[d] Calculated by difference.

shown by the calculated ΔE values of 3.8 and 30.5 for WPI and WPC-80, respectively, using the *L*, *a*, and *b* values of LVWPI as reference (Table 1.2).

The large ΔE value for the WPC-80 solution may be attributed mainly to its much higher *L* value compared with that of LVWPI. This may be explained by the considerable amount of fat in WPC-80 as well as the presence of protein aggregates in

Table 1.2. Color parameters at 20 °C of LVWPI and solutions of commercial WPI and WPC-80 containing 23.7% WP.

Parameter	Standard	LVWPI	WPI	WPC-80
L^a	95.543	26.704 ± 0.081	22.999 ± 0.203	55.794 ± 0.103
a^a	-0.238	0.244 ± 0.021	-0.462 ± 0.032	3.371 ± 0.032
b^a	0.590	4.444 ± 0.018	0.056 ± 0.074	0.014 ± 0.043
ΔL			-3.705 ± 0.227	29.090 ± 0.115
Δa			-0.706 ± 0.034	3.127 ± 0.035
Δb			0.240 ± 0.06	30.500 ± 0.050
$\boldsymbol{\Delta E}^b$			**3.8 ± 0.2**	**30.5 ± 0.1**

[a] Mean of triplicates ± standard deviation.

[b] $\Delta E = \sqrt{(L - L_{LVWPI})^2 + (a - a_{LVWPI})^2 + (b - b_{LVWPI})^2}$ where: *L*, *a*, *b* are the values obtained for WPI or WPC-80 solutions.

this product as indicated by our native PAGE results (data not shown). The ΔL valuefor the WPI solution indicates that it was slightly darker than LVWPI, which was probably due to the slight difference in the composition of these products. The negative *a* value of WPI solution indicated green hue, suggesting that WPI may still contained trace amounts of riboflavin, which is known to impart whey its greenish color (Walstra & Jenness, 1984).

The fresh LVWPI's density and viscosity at 20 ºC were 1.11 g cm^{-3} and 11.7 mPa s, respectively. From 10 to 50 °C, reconstituted freeze-dried LVWPI solution of the same WP concentration as fresh LVWPI had the same apparent viscosity profile as fresh LVWPI ($p \leq 0.05$) (Figure 1.2). Therefore, the measured viscosities of the

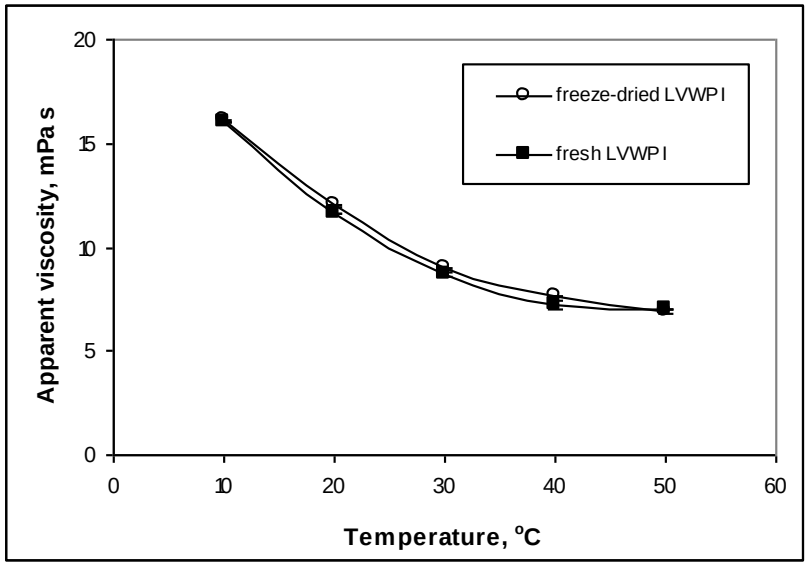

Figure 1.2. Variation of apparent viscosity (122.3 s^{-1} shear rate) with temperature (10-50 ºC) of freeze-dried LVWPI solutions and fresh LVWPI (within two days after production) of the same whey protein concentration of 23.7% (w/w). The error bars on the data, which are based on standard deviation from the mean of three trials, are not visible due to small standard deviations.

freeze-dried LVWPI solutions of different protein concentrations represented those of fresh LVWPI. The difference in the flow properties of freeze-dried LVWPI solutions,or simply LVWPI, and those of solutions prepared from commercial WP powders can be attributed to both the manner by which VW was recovered by MF before cheesemaking and concentrated without spray drying.

Figure 1.3 shows the variation of the apparent viscosities of WPI, WPC-80 and LVWPI containing 5 to 25% (w/w) WP at 10 and 50 ºC. The viscosities of LVWPI were consistently lower than those of WPI and WPC-80 solutions, which were comparable with values published previously by Hermannson (1975), Tang, Munro and McCarthy (1993), Rattray and Jelen (1995), Morison and Mackay (2001), and Bazinet, Trigui, and Ippersiel (2004).

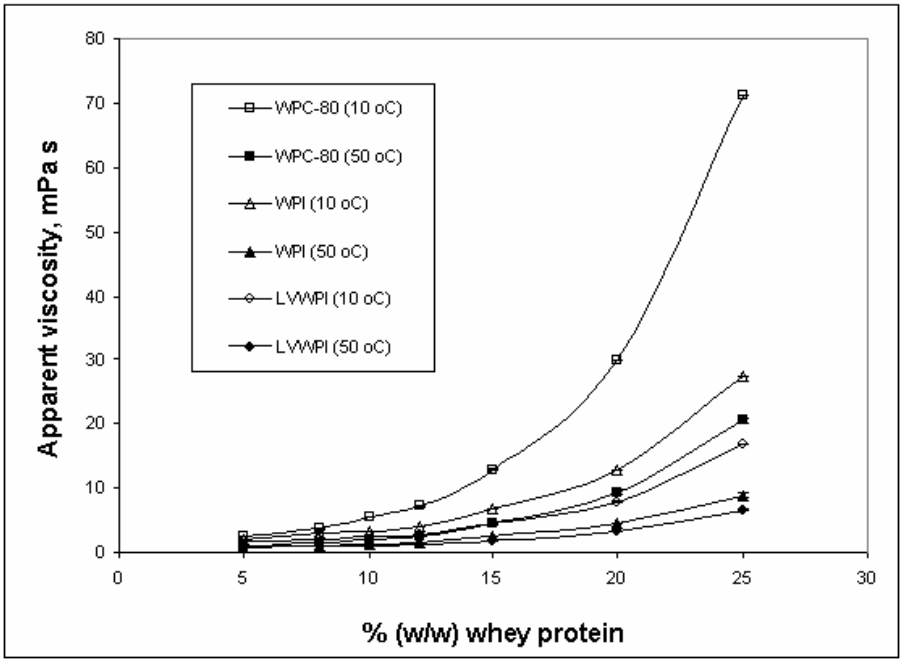

Figure 1.3. Variation of apparent viscosity (122.3 s^{-1} shear rate) with whey protein concentration for WPC-80, WPI and freeze-dried LVWPI solutions at 10 and 50 °C. Error bars are based on standard deviation from the mean of three trials from different batches of samples prepared.

Near neutral pH and at low ionic strength, native WP solutions exhibit low viscosities at ambient conditions (Rattray *et al*., 1995). Their relatively low molecular weight and spherical shape are responsible for the native WP's generally low viscosity in dilute solutions (Hermansson, 1975; Vardhanabhuti *et al*., 1999). At high WP

concentration, however, the proximity of the protein molecules increases, promoting intermolecular interactions that give rise to viscosity increase (Rattray *et al*., 1995). In the case of denatured globular proteins, the amino acid groups that are capable of forming hydrogen bonds in solution make better contact with the aqueous phase, increasing their water binding capacity (Schimdt, Packard, & Morris, 1984). As a consequence, swelling, an increase in the molecular radii of protein molecules, and greater molecular entanglements occur as the amount of denatured proteins increases (Rattray *et al*., 1995). This eventually results to higher solution viscosity. Although other components, such as lactose, contribute significantly to the viscosity of whey solutions, the influence of the proteins dominates (Morison *et al*., 2001). In this study, since WPI and LVWPI had almost equal amount of lactose (dry basis), the lower viscosity exhibited by the LVWPI suggests that it had greater amount of native WP.

1.4.3. Protein concentration and temperature effects on apparent viscosity

Results show that the LVWPI viscosity increased significantly with increase in WP concentration at all temperatures studied (Figure 1.4). Temperature did not significantly affect the apparent viscosity of LVWPI ($p \leq 0.05$) from 5 to 12% (w/w) WP. In this range, the maximum deviation from the mean was 0.35 mPa s at 10 °C across each concentration level. The effect of temperature became more pronounced above this concentration range. The WPI and WPC-80 solutions exhibited similar behavior except that their apparent viscosities were consistently higher, with the WPC-80 solutions having the highest viscosities. Greater amounts of lactose and fat, both have been found to considerably increase solution viscosity (Buma, 1980; Polyanskii & Rodionova, 1991), and the larger fraction of denatured WP in WPC-80 most likely contributed to this behavior.

Between 5 and 12% (w/w) WP concentration, the viscosity of the LVWPI varied linearly with concentration. At 15% (w/w) WP, this concentration dependence became increasingly non-linear. Using MATLAB® 7.0.4 software, a general equation was derived to describe the dependence of LVWPI viscosity on both concentration and temperature:

$$\eta = 0.0048\beta C^{\alpha} \exp\left[\frac{2164 * C^{0.051}}{T}\right] \qquad (1.1)$$

For T = 10 to 50 ºC:

$0.05 \leq C \leq 0.12$	$\alpha = 0.25$, $\beta = 1.00$	(error $\leq$ 5%)
$C = 0.15$	$\alpha = 0.25$, $\beta = 1.41$	(error $\leq$ 5%)
$0.20 \leq C \leq 0.25$	$\alpha = 2.28$, $\beta = 139.9$	(error < 10%)

where, η = apparent viscosity (mPa s), T = absolute temperature (K), C = weight fraction of WP, α is the *VW protein concentration parameter*, and β is the *LVWPI viscosity parameter*, which is a measure of the contribution to viscosity change of factors other than temperature and WP concentration, primarily molecular interactions. The estimation errors were calculated as % relative error between the predicted and the measured viscosities. The viscosities predicted by equation (1.1) and the actual viscosity data are shown in Figure 1.4.

Equation (1.1) suggests that concentration is the most dominant factor that affect LVWPI viscosity. The changes in the values of α and β suggest that, between 5 and 12% (w/w) WP, only C and T affect viscosity and that the influence of molecular interactions was negligible, a well-known characteristic of a dilute solution (Kasaai, Charlet, & Arul, 2000). At 15% WP, with α remaining the same, an increase in β value from 1.00 to 1.41 indicates that, other than changes in C and T, factors such as

weak intermolecular interactions among the proteins contributed to viscosity increase. The interactive volumes of the protein molecules, which are effects of both hydrodynamic and molecular interactions, would have overlapped to significantly

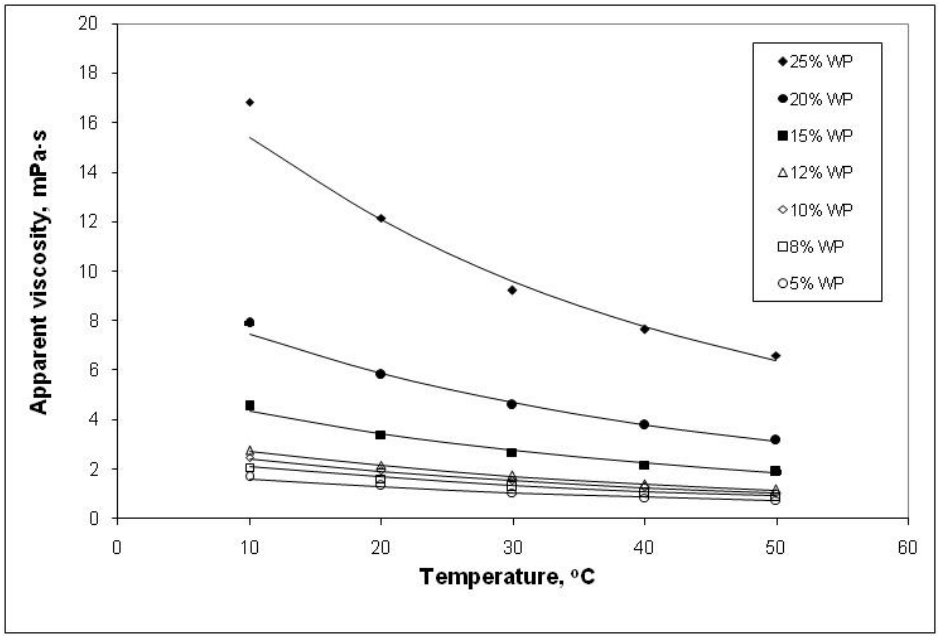

Figure 1.4. Variation of LVWPI apparent viscosity (122.3 s^{-1} shear rate) with temperature at different WP concentrations. The curves are model simulations and the points are experimental data. The error bars on the data, which are based on standard deviation from the mean of three trials, are almost invisible due to small standard deviations.

increase viscosity, and the solution may no longer be considered as dilute (Rha & Pradipasena, 1986; Tang *et al*., 1993; Kassai *et al*., 2000).

At WP concentrations higher than 15%, β increased sharply from 1.41 to 139.9, indicating more substantial molecular interactions among the protein molecules. The accompanying increase in α is an indication that there was a *critical concentration* where the LVWPI changed from a dilute solution to "semi-dilute" (Rha *et al*., 1986) and that at about 20% WP, that critical concentration may have been surpassed.

Since proteins in dilute solutions do not interact with each other, each molecule being kinetically independent from other molecules in the solution (Rha *et al.*, 1986), then our findings indicate that between 10 and 50 °C, the *dilute region* for LVWPI extended up to 12% WP. The small increase in β and the fact that α remained constant at 15% WP concentration indicate that the critical concentration of LVWPI is 15% WP or 16.5% TS.

Using Einstein's viscosity equation for spherical particles in dilute solution as model, with the assumption that weight fraction was directly proportional to volume fraction, Tang *et al*. (1993) found that at 22 °C and pH 7, the linear dependence of apparent viscosity with concentration for WPC-80 solutions extended only up to 8% TS. They surmised that such behavior is due to the absence of considerable molecular interactions up to 8% TS and suggested this to be the critical concentration of WPC-80.

Our findings are similar to those of Morison *et al*. (2001), who used both fresh cheddar cheese whey UF retentates and reconstituted WPC-80 powder to study the influence of WP and lactose concentrations on solution viscosity. They suggested that at 20 °C, the Einstein equation works well up to 15% protein concentration, above which intermolecular forces affect viscosity. They proposed an empirical equation to estimate viscosity, which was a power law dependence of apparent viscosity with protein and lactose fractions in the solution. Since these authors used WPC-80, which contained considerable amounts of fat and lactose, the viscosity values they obtained at similar WP levels as LVWPI in the present work were higher.

Native globular proteins have small effects on the viscosity of water in dilute solutions (Rha *et al*., 1986). This attribute of native globular proteins and the high critical concentration exhibited by the LVWPI indicate that the WP it contained were mainly in their native form.

1.4.4. Activation energy of flow

Our results indicate that LVWPI and the commercial WPC-80 and WPI solutions satisfactorily followed the Arrhenius model for temperature dependence (Table 1.3) with R^2 values of at least 0.98. The E_A value of 20 kJ mol^{-1} at around 20%

Table 1.3. Activation energy of flow using the Arrhenius model for LVWPI, WPC-80 and WPI solutions at pH 6.1.

%WP	E_A, kJ·mol^{-1}		
	WPC-80	WPI	LVWPI
5[a]	18.50	17.15	15.77
8[a]	18.95	17.65	16.07
10[a]	19.24	17.74	16.32
12[a,b]	19.79	18.17	16.47
15[b]	19.94	18.60	16.82
20[b]	21.86	20.32	17.25
25[b]	23.76	21.27	17.84

[a] Viscosity (mPa s = centistokes x sp. gravity) measured in triplicates using Canon-Fenske 75 viscometer.
[b] Viscosity (mPa·s) measured in triplicates using Brookfield DV-II viscometer equipped with UL adapter.

TS (w/w) obtained for WPI and WPC-80 solutions in this work were comparable with those obtained by Tang *et al*. (1993) and Morrison *et al*. (2003). Interestingly, at the WP concentrations studied in the present work, the LVWPI viscosity changes were less sensitive to temperature changes as indicated by their smaller E_A values compared with those of the commercial products. The differences in the E_A values of LVWPI as WP concentration increased were also small. From 15 to 25% (w/w) WP

concentration, average differences in E_A values were only 0.46 kJ·mol^{-1} while those of WPI and WPC-80 solutions were 1.03 and 1.32 kJ mol^{-1}, respectively.

Higher E_A values indicate more rapid change in viscosity with temperature (Steffe, 1996) while high differences in E_A values at different solute concentrations are indicative of high-energy barrier to viscous flow (Krokida, Maraoulis, & Saravacos, 2001; Herceg *et al.*, 2005). Both are observed when temperature changes bring about considerable solute interactions manifested by viscosity changes, which in the case of globular proteins in aqueous solution are more likely to take place when the proteins are unfolded (Rha *et al*., 1986; Herceg *et al.*, 2005). Thus, our results suggest that the intermolecular interactions in LVWPI between 15 and 25% WP are the weakest compared with both WPI and WPC-80. Such weak intermolecular interaction, even at high protein concentration, indicates high mobility of the proteins in solution, which in turn suggests that the proteins are in their native globular conformation (Kinsella, 1984; Rattray *et al.*, 1995).

Tang *et al*. (993) and Morison *et al.* (2001) studied the variation of the E_A of WPC-80 solutions as a function of concentration from 5 to 60 °C and from 10 to 50 °C, respectively. Their data showed quadratic dependence of E_A with TS content. Morison *et al.* (2001) suggested that the E_A equation they obtained be used together with the empirical viscosity equation they obtained at 20 °C and the Arrhenius equation to estimate solution viscosities at a given combination of temperature and WP concentration. In the present work, using only equation (1), the viscosity of LVWPI may be estimated at a specified temperature and WP concentration from 10 to 50 °C and 5 to 25% (w/w) WP.

1.4.5. Effect of heat treatment on LVWPI apparent viscosity

To confirm that the unique viscosity profile exhibited by LVWPI was due to its native WP content, its viscosity profile after heat treatment at 65 °C for 2 minutes was obtained between 10 and 50 °C. The same treatment was done to WPI and WPC-80 solutions. Interestingly, the viscosity profile of heat-treated LVWPC was found to be statistically identical ($p \leq 0.05$) with that of unheated WPI solution (Figure 1.5). This suggests that the heat-treated LVWPI had the same amount of denatured WP as the

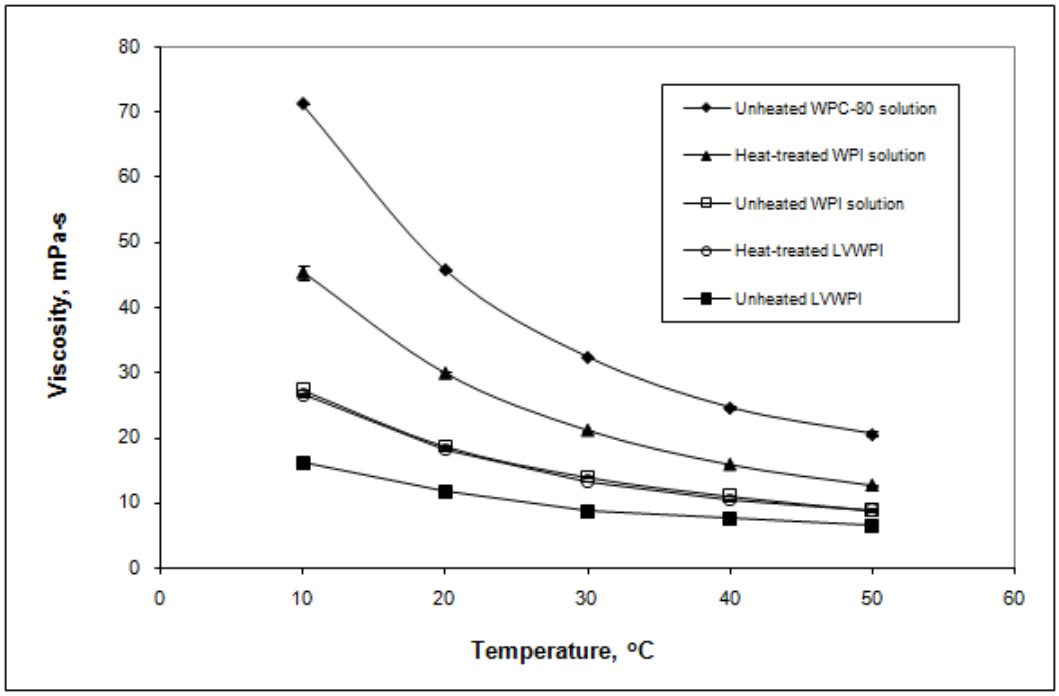

Figure 1.5. Comparison of the apparent viscosities (122.3 s^{-1} shear rate) of unheated and heat-treated whey protein solutions containing 23.7% (w/w) WP. The error bars on the data, which are based on standard deviation from the mean of three trials, are almost invisible due to small standard deviations.

unheated WPI solution, indicating that the LVWPI is richer in native WP than the commercial WPI. DSC analyses showed that there was about 18% decrease in the enthalpy of denaturation of LVWPI (results not shown) after heat treatment. This is in

agreement with the finding of Fachin *et al.* (2005) who reported reduction of WP solubility at pH 6 in fresh UF retentates from sweet whey after being subjected to heat treatment of 68 ºC for 2 minutes, an indication of fractional denaturation. The heat-treated WPC-80 solution in the present study turned to gel upon cooling following heat treatment, therefore, its viscosity was not shown in Figure 1.5.

1.5. CONCLUSION

A two-stage UF with DF system was used to produce LVWPI from VW, which was harvested by MF of skim milk at pH 6.0 prior to cheesemaking. The operatingparameters and conditions employed in the UF system gave improved permeate flux values compared with literature data on UF of cheese whey using PSf membrane in spiral wound and hollow fiber configurations. The LVWPI was a clear, slightly brown, liquid containing 26.13 % TS (w/w), about 91% of which was WP. The results of this study suggest that the unique physicochemical properties of LVWPI were rendered by the greater amount of native WP it contained compared with commercial WP products, which were produced from cheese whey and spray dried following UF concentration. Having been produced from VW, which was recovered by MF prior to cheesemaking, concentrated by UF alone, and not having to undergo spray drying all contributed to the unique physicochemical behavior exhibited by LVWPI.

1.6. ACKNOWLEDGMENTS

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CHAPTER 2

Functional behavior of liquid virgin whey protein isolate

2.1. ABSTRACT

This study investigated the functional behavior of liquid virgin whey protein isolate (LVWPI) made by a two-stage membrane filtration process. LVWPI's enthalpy of denaturation was found to be 11-25% higher than those of commercial whey protein isolate (WPI) and concentrate (WPC-80) manufactured from sweet whey by microfiltration (MF) and ultrafiltration (UF). Confocal laser scanning microscopy images showed that LVWPI gels were made of strands, thicker than those found in gels made from WPI manufactured from sweet whey by ion-exchange (IE WPI), and smaller aggregates compared to those of MF-UF WPI gel. Because of LVWPI's mineral profile, adding 5 mM $CaCl_2$ increased the storage modulus (G') of LVWPI gel to equal that of commercial MF-UF WPI gel without $CaCl_2$ added to it, while the addition of 10 mM $CaCl_2$ to both LVWPI and IE WPI increased their G' to equal values, at the same protein concentration. These indicate LVWPI's potentials as food ingredient for fine-tuned texturization.

2.2. INTRODUCTION

Whey proteins (WP) are widely utilized in food formulation because of their desirable functional properties such as solubility, foaming, emulsification, water binding and retention, dispersability, viscosity, turbidity, and heat-induced gelation and coagulation (de Wit, 1984; Morr & Ha, 1993; Havea *et al.*, 1998). These functional properties are manifestations of their hydrodynamic and surface-related physicochemical properties, and are strongly influenced by WP product composition and the processing protocols used during manufacture (Morr & Ha., 1993).

The ability of WP to form gel after heat-induced or pH-induced denaturation makes them useful texture enhancers in food applications (de Wit *et al*., 1983; Dybing & Smith; 1991; Morr & Ha., 1993; dela Fuente *et al*., 2002). The gelation process involves the physical and chemical transformations of one type of structure into another (Caussin *et al*., 2003). These structures are the denatured conformation of the proteins and the aggregated or polymerized form that eventually makes a space-filling structure that is the gel (Verheul & Roefs, 1998). These transformations depend on many factors, such as protein concentration, the physicochemical environment, and the extent of protein denaturation, all of which depend on the method by which WP are obtained and processed (de Wit, 1984; Schimdt *et al*., 1984; Mahmoud, *et al*., 1990; Patel *et al*., 1990; Hollar *et al*., 1995).

Over the years, the food industry has developed various commercial-scale processes for manufacturing highly functional whey protein concentrate (WPC) and isolate (WPI) with nutritional, functional and sensory qualities suitable to various food applications (de la Fuente *et al*., 2002). However, cheese manufacturing methods cause compositional differences in cheese whey while the changes in the physicochemical conditions during cheesemaking and heat applications during processing affect the native conformation of WP and alter their functional properties

(Singh & Havea, 2003). These result in highly variable and unpredictable functional behavior of commercial WP and impede attainment of the desired quality in food products (de Wit & Klarenbeek, 1984; Schimdt, *et al*., 1984; Manji & Kakuda, 1987; de Wit, 1990; Patel *et al*., 1990; Singh & Havea, 2003). In cases where WP aggregation and/or gelation are/is relied on for texture development, texture uniformity and fine-tuning are the common challenges. Therefore, harvesting and concentrating native WP to a product that's compositionally consistent and of predictable functional behavior may enable fine-tuned texturization in specific food products.

The liquid virgin whey protein isolate (LVWPI) is a native protein-rich ingredient with physicochemical properties not observed in commercial WP products manufactured from cheese whey, such as low viscosity and density even at concentration as high as 15% (w/w) and/or low temperature of 10 °C (Marcelo & Rizvi, 2008). It is produced by concentrating "virgin whey" (VW) by two-stage ultrafiltration (UF) with diafiltration (DF) (Marcelo & Rizvi, 2008). Harvested before cheesemaking as permeate from the microfiltration (MF) of skim milk at pH 6, VW is compositionally invariant with the type of cheese subsequently made (Ardisson-Korat & Rizvi, 2004). Also, since it is microbially sterile and does not contain cheesemaking remnants, VW does not require any pre- treatment prior to concentration (Brandsma & Rizvi, 1999).

It was previously shown through comparative physicochemical properties analyses with commercial WPC-80 and WPI, produced from sweet whey and concentrated by UF, that LVWPI contained greater amount of native WP (Marcelo & Rizvi, 2008). However, whether the unique physicochemical properties exhibited by LVWPI translate to unique functional behavior, specifically aggregation and gelation, was not established. The objective of this study was to investigate the heat-induced

gelation behavior of LVWPI. Since gel structure and properties are related to the thermal properties and aggregation of the proteins, both were studied together with the effect of spray drying on LVWPI's thermal properties. Also, since salt composition is another important determinant of functional properties of WP products, the gelation behavior of LVWPI with and without the addition of $CaCl_2$ was studied and compared with those of the commercial products.

Marcelo and Rizvi (2008) showed previously that there was no significant difference in the dry-basis composition and flow properties of fresh LVWPI and reconstituted freeze-dried LVWPI. Therefore, reconstituted freeze-dried LVWPI was used in studying the functional behavior of LVWPI. To illustrate how LVWPI's functional behavior was different from other products, commercial WPC-80 and WPI (MF-UF WPI), which were both manufactured from sweet whey and concentrated by membrane technology before spray drying, were used for comparison. These products were chosen because, like LVWPI, they were processed by membrane technology, but unlike LVWPI, they were made from whey that was recovered from conventional cheesemaking. Although the functional behavior of whey protein products depends on a number of factors, in this study, differences in functional behavior could be attributed primarily to the manner by which whey was recovered. In addition, a second commercial WPI sample manufactured by ion-exchange (IE WPI) was used in the gelation studies to illustrate how LVWPI's functional behavior compares with that of a product concentrated and purified by method other than membrane technology.

2.3. MATERIALS AND METHODS

2.3.1. Whey protein samples

LVWPI was produced using VW from the vatless manufacture of cheese as described by Ardison-Korat and Rizvi (2004). The VW was concentrated and purified

to produce LVWPI using a pilot-scale two-stage UF/DF system in a process detailed elsewhere (Marcelo & Rizvi, 2008). Two batches of 1000 g LVWPI obtained from two pilot plant runs were freeze-dried at 5 μmHg vacuum and -85 °C using Labconco bench-top freeze-drier (Kansas City, MO, USA). The dried LVWPI was ground to approximately 100 μm particle size. To determine the effect of spray drying on LVWPI thermal properties, 1000 g of fresh LVWPI was spray dried using Yamamoto Pulvis Basic Unit Model GB-21 (Yamamoto Scientific Co., Ltd. Tokyo, Japan) bench top spray drier with inlet and outlet air temperatures of 190 and 90 °C, respectively. Commercial WPC-80 and MF-UF WPI (Glanbia Nutritionals, Monroe, WI, USA) that were both manufactured from sweet whey and concentrated by membrane technology prior to spray drying were used for comparison. A second commercial WPI sample, an IE WPI (Davisco Foods Davisco Foods International, MN, USA) was used in the gelation studies.

2.3.2. Compositional analyses

The dried samples were analyzed of their gross composition. The %TS was determined by drying in an oven at 100 °C for four hours (AOAC, 2000; 33.2.44, 990.20). Total nitrogen was determined by Kjeldahl method (AOAC, 2000; 33.2.11, 991.20) and the true protein was obtained after correction for non-protein nitrogen (NPN) (AOAC, 2000; 33.2.12, 991.21) using a protein conversion factor of 6.38. Fat was analyzed using Mojonnier extraction procedure (AOAC, 2000; 33.2.26, 989.05). The ash content was determined by ashing the samples at 550 °C in an electric muffle furnace. Lactose was calculated by difference between the TS and other solid components. All determinations were done in quadruplicates. The same analyses were done on the commercial MF-UF products. The mineral profile of freeze-dried LVWPI ash was also determined by Inductively Coupled Plasma-Atomic Emission

Spectroscopy (ICP-AES) (ICAP 61E. Thermal Jarell Ash Trace Analyzer, Jarell Ash Co., Franklin, MA, USA).

2.3.3. Preparation of whey protein solutions

Appropriate amounts of WPC-80, WPI, and freeze-dried and spray dried LVWPI powders were dissolved in 50 mL of deionized water to make 1, 8 and 10% protein (w/w) dispersions as described by Morr *et al*. (1985). After stirring for an hour, the dispersions were allowed to equilibrate for 30 min, checked for undissolved particles and then stirred again for 30 min. The dispersions were then allowed to equilibrate overnight at 4 °C. Before analysis, the pH of the solutions were measured and adjusted to 6.0 ± 0.1 using 0.1 M NaOH or HCl as appropriate. The pH adjustment was made in order to characterize LVWPI at its "natural pH", meaning its pH from manufacture, which was about 6.0, and to compare it with the commercial products at the same pH. In the gelation studies, appropriate amounts of WPC-80, MF-UF WPI, IE WPI and LVWPI were dissolved to make 8% protein (w/w) solutions in deionized water as described above, and appropriate amounts of $CaCl_2$ were added to make solutions of 5 mM and 10 mM $CaCl_2$ concentration.

2.3.4. Polyacrylamide gel electrophoresis

The samples, containing 1% protein in deionized water and adjusted to pH = 6.0 ± 0.1 with 0.1 N HCl or 0.1 N NaOH, were analyzed using the Bio-Rad Laboratories (Hercules, CA) Mini-Protean II dual slab system. Tris-HCl gel having 12% resolving gel and 4% stacking gel was used to resolve the protein fractions. A standard with pre-stained protein bands ranging from 10 kDa to 250 kDa was used as reference. 5X electrode buffer (9 g Tris base, 43.2 g Glycine, and 3 g SDS diluted to 600 mL with deioized water) diluted with deionized water 1:4 as per Laemmli's

(1970) method was used. The samples were diluted with SDS reducing buffer composed of deionized water, 0.5 M Tris-HCl (pH 6.8), glycerol, 10% (w/v) SDS, 2-mercaptoethanol and 1% (w/v) bromophenol blue by 1:7.5 (Laemmli, 1970). The samples were heated at 95°C for four min using constant-temperature water bath. 10 μL of each dilute sample was injected to the gel sample well. Electrophoresis was run at constant voltage of 200 and an initial current of 60 mA per gel. After running, gels were stained for 24 hours using Coomassie blue staining buffer. Following staining, the gels were destained with multiple changes of 40% methanol, 10% glacial acetic acid solution. The relative intensity of stained bands in the gels was then analyzed using the Epi Chemi II Darkroom with UV Transmittor (UVP, Inc., Upland, CA, USA) equipped with Labworks Image Acquisition and Analysis Software (UVP, Inc., Upland, CA, USA).

2.3.5. Thermal properties

Differential scanning calorimetry

The thermal properties of 10% (w/w) protein solutions of both freeze-dried and spray dried LVWPI, WPI and WPC-80 were determined using DSC Q10 (TA Instruments, New Castle, DE, USA) differential scanning calorimeter (DSC) equipped with TA Universal Data Analysis (TAUDA) software. The instrument was first calibrated for temperature and cell constant using indium as a standard. Baseline calibration was also conducted with the cell empty. For each sample, 5 ± 0.3 mg of the protein solution, prepared as described previously, was dispensed in a pre-weighed aluminum pan with lid. The covered pan was sealed using an encapsulating press and then weighed once more to verify the exact weight of the sample in the capsule. The sample was then scanned between 20 and 120 °C at a scanning rate of 2 °C min^{-1} using deionized water of the same weight as a reference. The scanned sample was

cooled to room temperature, re-weighed and then re-scanned to ensure the complete denaturation of the proteins in the sample. Only samples with 5% or less change in weight before and after scan were considered for analysis. The onset of denaturation temperature, T_o, and the denaturation temperature, T_D, were determined from the thermogram using TAUDA software. The peak maximum temperature was taken as T_D, and the temperature at the extrapolation of maximum deflection of the curve onto the baseline was taken as T_o. The TAUA software also enabled the calculation of the enthalpy of denaturation, ΔH, expressed in J per g of sample, employing the Borchardt-Daniels method. The calculated ΔH was used to determine the enthalpy of denaturation as J per g of protein in the sample. Analyses were done in quadruplicates for every sample.

2.3.6. Aggregation

Viscosity measurements

LVWPI, WPI and WPC-80 solutions with 8% (w/w) protein were prepared as described previously. The temperature of the solutions was adjusted from room temperature to 70 ºC at 5 ºC min^{-1} heating rate and at 5 s^{-1} shear rate. Upon reaching 70 ºC, the viscosity of each solution was then measured continuously for three hours at 244 s^{-1} shear rate using Brookfield DV-II viscometer (Middleboro, MA, USA) equipped with UL adapter and Wingather software for flow analysis. The viscosity was recorded at 30-s intervals. Measurements were done in triplicates for each sample. The rate of aggregation was related with the rate of change in viscosity within the test period.

2.3.7. Gelation

Rheological analysis

A small-strain test was carried out on 8% (w/w) protein solutions using 50 mm-diameter parallel plate and base configuration with a 1.0 mm gap set-up in Advanced Rheometer Expansion System (ARES) rheometer (TA Instruments-Waters LLC, New Castle, DE, USA) with TA Orchestrator software and SR5 PELTIER Circulator for temperature maintenance. The solid-like properties of the heated solutions were determined and compared by continuously recording the storage (G') modulus at a fixed frequency of 0.5 Hz using constant stresses (producing strains up to 2%) within the range of linear viscoelastic behavior determined from stress sweeps performed for each protein preparation at 80 ºC and after cooling to 25 ºC. Protein solutions were loaded between the plate and base, adding a few drops of mineral oil to cover the edge of the plate and prevent evaporation. The solutions were equilibrated at 25 ºC for 5 min, heated from 25 to 80 ºC at 5 ºC min^{-1} heating rate, held at 80 ºC for 30 min, cooled to 25 ºC at 2 ºC min^{-1} cooling rate, and held at 25 ºC for 30 min. Frequency sweeps (0.01 – 100 rad s^{-1}) were performed after cooling to confirm gel network formation. Measurements were done in triplicates for every sample.

Confocal laser scanning microscopy

Gels made from solutions of 10% (w/w) protein concentrations, heated at 80 °C for 10 min and stained with the non-covalent dye Rhodamine B at 1:10 volumetric ratio, were observed using Leica TCS SP Confocal Laser Scanning Microscope (CLSM) (Bannockburn, IL, USA). Images were taken using 40x magnification dry objective. Confocal illumination was provided by a Krypton/Argon laser (488 nm and 568 nm excitation).

2.3.8. Statistical analyses

All statistical analyses were done using MINITAB® release 14 statistical software (State College, PA, USA).

2.4. RESULTS AND DISCUSSION

2.4.1. LVWPI composition

The composition of freeze-dried and spray dried LVWPI powders, and the commercial WP products are shown in Table 2.1. The dry-basis composition of both freeze- and spray dried LVWPI and MF-UF WPI were similar, except that fat was not

Table 2.1. Gross composition of freeze-dried LVWPI, spray dried LVWPI, and commercial MF-UF WPI and WPC-80.

Component	**% Composition (w/w)**			
	This study		Commercial products	
	Freeze-dried LVWPI	Spray dried LVWPI	MF-UF WPI	WPC-80
Total solids[a]	99.29 ± 0.01	95.22 ± 0.07	95.30 ± 0.20	95.10 ± 0.26
True protein[a,b]	90.10 ± 0.19	89.35 ± 0.25	89.66 ± 0.13	81.94 ± 0.34
NPN[a,b,c]	5.13 ± 0.11	5.19 ± 0.02	4.83 ± 0.13	2.08 ± 0.10
Fat[a,b]	0.00 ± 0.00	0.00 ± 0.00	0.79 ± 0.04	6.68 ± 0.12
Lactose[a,b,d]	1.69 ± 0.45	2.47 ± 0.20	1.60 ± 0.09	6.58 ± 0.16
Ash[a,b]	3.08 ± 0.02	2.99 ± 0.03	3.12 ± 0.09	2.73 ± 0.08
Water[a]	0.71 ± 0.01	4.78 ± 0.07	4.76 ± 0.14	4.90 ± 0.08

[a] Mean of quadruplicates ± standard deviation.
[b] Dry basis.
[c] Non-protein nitrogen.
[d] Calculated by difference.

detected in LVWPI. The SDS-PAGE analysis (Figure 2.1) shows that the four major WP: β-Lactoglobulin (β-Lg), α-Lactalbumin (α-La), bovine serum albumin (BSA) and immunoglobulin, were present in both the freeze-dried and spray dried LVWPI in similar proportions as in commercial MF-UF WPI. β-Lg was present in the largest proportion in all three samples. There was no pronounced difference between the freeze-dried and spray-dried LVWPI. Figure 2.2 shows the SDS-PAGE patterns for the WPC-80 sample in comparison with LVWPI, where WPC-80 was found to contain trace amounts of β-casein.

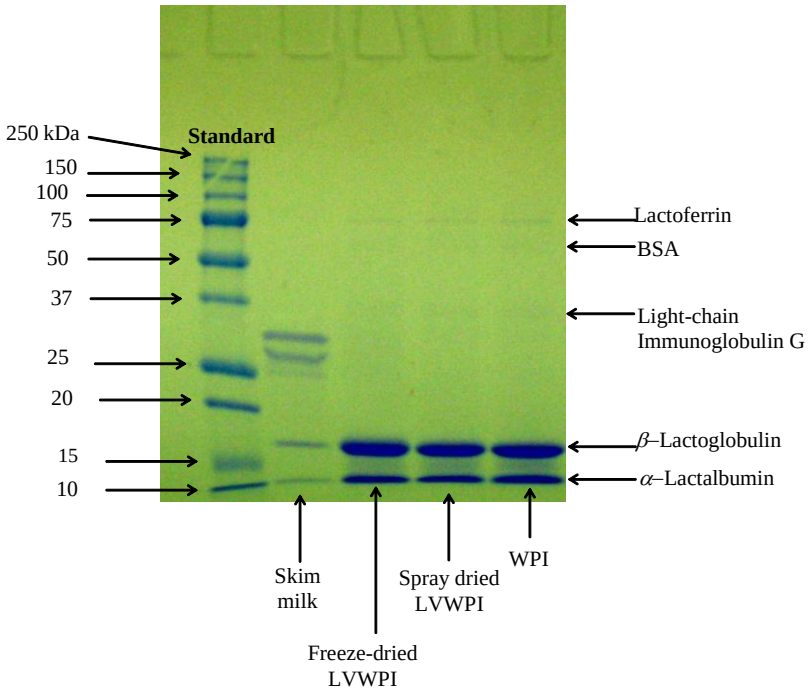

Figure 2.1. SDS-PAGE patterns of skim milk, freeze-dried LVWPI, spray dried LVWPI and commercial WPI at pH 6.0 ± 0.1.

2.4.2. Thermal properties

When heat-sensitive globular proteins, such as WP, are heated in an aqueous medium, they unfold cooperatively to random coil conformation or to conformation

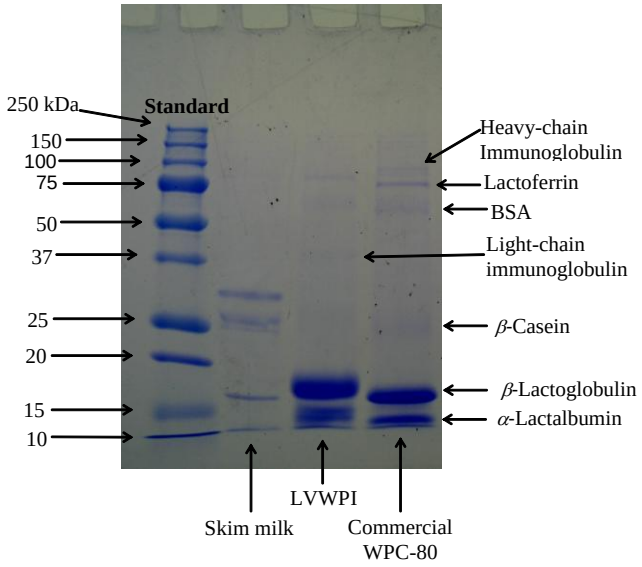

Figure 2.2. SDS-PAGE patterns of skim milk, freeze-dried LVWPI and commercial WPC-80.

close to random coil (Tanford, 1961; Paulsson & Dejmek, 1990). This heat-induced unfolding or heat-induced denaturation is accompanied by endothermic effects, which can be measured by DSC analyses (Sturtevant,1987; Paulsson & Visser, 2001).

The results of the DSC analyses (Table 2.2) show that there was no significant difference ($p \leq 0.05$) in the denaturation temperatures of WPC-80, MF-UF WPI and LVWPI samples. This indicates that the cooperative endothermal unfolding of the proteins into random coil conformation at pH 6.0 in all the samples took place at approximately the same temperature of about 81 °C. The similar denaturation temperatures of the products were expected as they all contain the major WP in similar proportions as shown by the SDS-PAGE results (Figure 2.1 and Figure 2.2).

The considerable amount of lactose in WPC-80 (Table 2.1) may have increased the onset of denaturation and denaturation enthalpy of WPC-80 compared with MF-UF WPI, as lactose is known to protect WP from denaturation, at the expense of reducing their biological activities (Morr & Ha, 1993; Spiegel, 1999).

Patel *et al*. (1990) reported that cheddar-cheese-type WPC at a pH of 6.36 exhibited a single broad endothermic peak with denaturation temperature near 76 °C, and enthalpy of denaturation ranging from 11.46 to 12.38 J per g protein, which were lower than those found for the commercial WPC-80 in the present study. Patel *et al*. (1990) explained that the variable enthalpy of denaturation they observed was due to varying sample composition.

Table 2.2. Thermal properties of LVWPI, MF-UF WPI and WPC-80 in 10% (w/w) protein solutions at pH = 6.0 ± 0.1.

Material	Onset of denaturation, °C[a]	Denaturation temperature, °C[a]	Enthalpy of denaturation, J per g protein[a]
Freeze-dried LVWPI	75.53 ± 0.25	82.97 ± 0.70[c]	22.15 ± 0.72[e]
Spray dried LVWPI	73.94 ± 0.72[b]	81.79 ± 0.45[c,d]	21.91 ± 0.36[e]
MF-UF WPI	67.50 ± 0.30	81.05 ± 0.42[d]	16.50 ± 1.32
WPC-80	73.83 ± 2.01[b]	81.70 ± 0.97[d]	19.58 ± 2.41

[a] mean of quadruplicates ± standard deviation.
[b, c, d, e,] difference is not statistically significant.

Interestingly, the onset of denaturation of the MF-UF WPI sample was at a considerably lower temperature of about 67.50 °C and it had a lower average enthalpy of denaturation of 16.50 J per g protein compared to the freeze-dried and spray dried LVWPI samples (Table 2.2). Previous investigations on the thermal behavior of WP indicate that heating at ≥65 °C causes denaturation and aggregation of WP (Morr & Ha, 1993). BSA and α-La are the WP fractions that were found to have low onset of denaturation of about 64 °C and 62 °C, respectively (de Wit, 1984; Kinsella, 1984), while β-Lg was found to be the most thermostable against denaturation owing to its

high level of β-sheet structure in the native state as compared with BSA and α-La (Paulsson & Visser, 1992; Prabakaran & Damodaran, 1997). Although α-La in pure form is most thermostable against aggregation because of its ability to renature on cooling, heat-induced interactions with denatured β-Lg and BSA leads to aggregation in WP solutions (de Wit & Klarenbeek, 1984). Aggregation, which is an irreversible reaction that follows protein denaturation, deters the establishment of equilibrium between the native and unfolded state of proteins by driving the denaturation reaction further even at constant temperature (Paulsson & Dejmek, 1990). During heating in the presence of denatured β-Lg, protein aggregation results in a shift of denaturation equilibrium which favors the conversion of native protein molecules to denatured molecules, and consequently further aggregation on heating (Hoffmann *et al*., 1996). Since aggregation is an exothermic process, it results in lower peak temperature and lower enthalpy of denaturation in DSC measurements (Paulsson & Dejmek, 1990; Ju *et al*., 1999). Using 10% protein solutions of IE WPI in a wide range of pH, Ju and Kilara (1998) reported that the transition of WP molecules to soluble aggregates takes place at pH 6.0, and that the rate of aggregation was aided by the addition of $CaCl_2$ and/or heating.

The DSC results in the present study suggest that aggregation may have taken place in the MF-UF WPI sample during DSC test. Considering that β-Lg and the other protein fractions were present in similar proportions in the MF-UF WPI and LVWPI samples as shown by SDS-PAGE, the observed differences in thermal properties of these products therefore may have been caused by differences in the initial molecular conformations of the major WP they contained and their mineral profiles.

By DSC analyses and using IE WPI in 10% protein (w/v) solution at pH of 6.2, Ju *et al*. (1999) observed two distinct onset of denaturation at 63.2 (presumably for α-

La) and 75.7°C (presumably for β-Lg), two endothermic peaks, which were taken as denaturation temperatures at 67.1 (presumably for α-La) and 80.1 °C (presumably for β-Lg), and a denaturation enthalpy of 3.59 J per g of sample, or about 35.9 J per g of protein. Although the onset of denaturation and denaturation temperatures observed in the present study were reasonably close to those reported previously, it is interesting to note that the measured enthalpy of denaturation of MF-UF WPI was less than half of that reported by Ju *et al*. (1999), while those of LVWPI were only about two-thirds of the IE WPI enthalpy reported by Ju *et al*. (1999). Since IE WPI is known to contain smaller amount of salt compared to MF-UF WPI (Morr & Ha, 1983), these differences in the denaturation enthalpies in the present study and that reported by Ju *et al*. (1999) on IE WPI indicate that the mineral profiles of the samples may have been a bigger factor compared with the effect of initial molecular conformation of the major WP.

2.4.3. Aggregation

The low enthalpy of denaturation exhibited by the commercial MF-UF WPI solution in the DSC analyses, which suggested that considerable aggregation may have taken place during the test, was investigated further by rheological analysis using solutions of 8% (w/w) protein concentration. Results show (Figure 2.3) that after about 10 minutes at 70 °C, which was near the onset of denaturation of all the samples, a rapid increase in the viscosity of the MF-UF WPI solution at about 0.17 mPa s per min occurred until a viscosity of about 24 mPa s was reached in 130 minutes, indicating occurrence of aggregation. After 130 minutes, the aggregates grew too big to continue the test at the experimental conditions in the viscometer. The LVWPI viscosity was constant for about 90 minutes before increasing to about 24 mPa s in 175 minutes at the rate of 0.30 mPa s per min. This confirmed the thermal stability of LVWPI against rapid aggregation, which was previously suggested by its

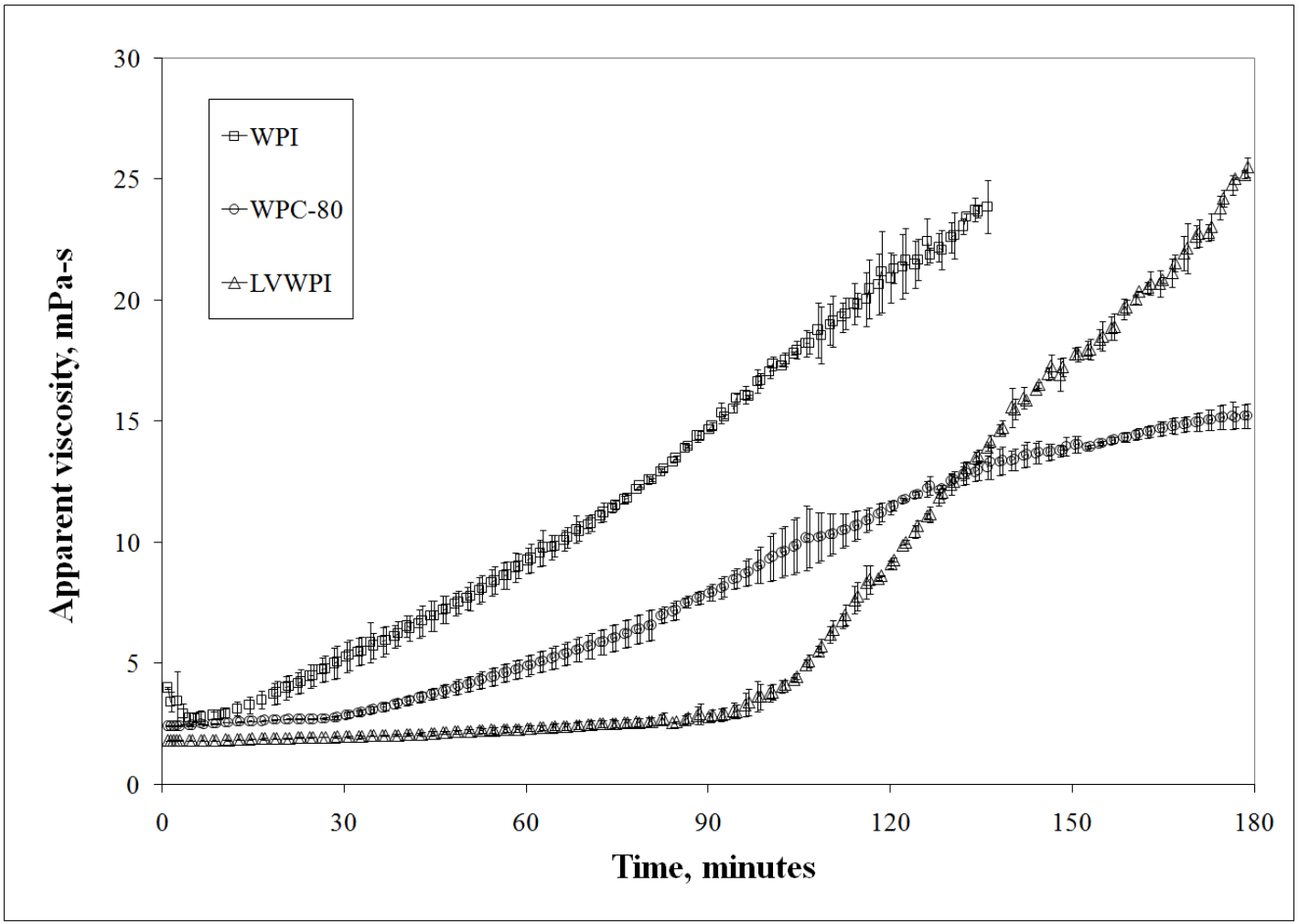

Figure 2.3. Changes in apparent viscosity with time for 8% (w/w) protein solutions at 70 °C and 244.6 s^{-1} shear rate. Error bars are based on standard deviation in three trials.

thermal properties. Imparted partly by its mineral profile, this time-delayed thermal stability suggests a potential opportunity to exploit modulated heat-induced aggregation and eventual gelation. The WPC-80 solution's viscosity also increased but only after about 30 minutes of heating at 70 °C. Compared with both LVWPI and MF-UF WPI solutions, WPC-80 showed a lower rate of viscosity increase and a lower final value of about 15 mPa s at the end of 180 minutes.

When the temperature in the viscosity analysis was raised to 80 °C, both MF-UF WPI and WPC-80 rapidly formed large aggregates and eventually turned to gel after less than 10 minutes of measurement. Although its viscosity increased significantly, the LVWPI solution did not turn to gel at the same test conditions. The increase in the viscosity was deemed to have been caused by the formation of soluble aggregates. Unlike the commercial products, however, the LVWPI aggregates did not associate to form a gel, indicating low content of minerals, such as Ca and Na, that aid gelation. Similarly, using atomic force microscope (AFM), Ikeda and Morris (2002) reported the formation of soluble aggregates of diverse shapes and sizes that did not form gel in an aqueous solution of IE WPI with 11% (w/w) protein concentration heated for 60 minutes at 80 °C and pH 7. The same authors observed more intensive protein aggregation at much lower protein concentration of 2% (w/w) in IE WPI solution at the same pH and heating conditions when 0.1M NaCl was added.

Findings from previous studies suggested that heat-induced WPI gelation near neutral pH occurs beginning in a two-step aggregation process. Following heat-induced denaturation, protein aggregation starts from the formation of "primary aggregates", which then grow into strands that eventually form the gel network (Hines & Foegeding, 1993; Roefs & de Kruif, 1994; Ikeda & Morris, 2002; Kazmierski & Corredig, 2003). At a pH sufficiently away from the isoelectric point, the initial aggregation kinetics is influenced by mineral content (Caussin *et al*., 2003). For this

reason, previous studies on WP aggregation were carried out using IE WPI, which contained lower amount of total minerals than MF-UF WPI (Morr & Ha, 1993), to allow the modulation of thermal aggregation by altering mineral content as appropriate. Considering that LVWPI behaved more like IE WPI than MF-UF WPI, we proceeded to look at its mineral profile and to investigate its gelation behavior with and without the addition of $CaCl_2$.

2.4.4. Gelation

The solid-like behavior of the protein solutions after heating at 80 °C for 30 minutes was determined by recording G' in small-strain rheological test. All solutions exhibited tan $\delta \leq 0.2$, indicating that storage modulus was greater than the loss modulus and thus of more solid-like behavior. The G' values of the MF-UF WPI solutions were greater than those of both WPC-80 and LVWPI (Figure 2.4). As the

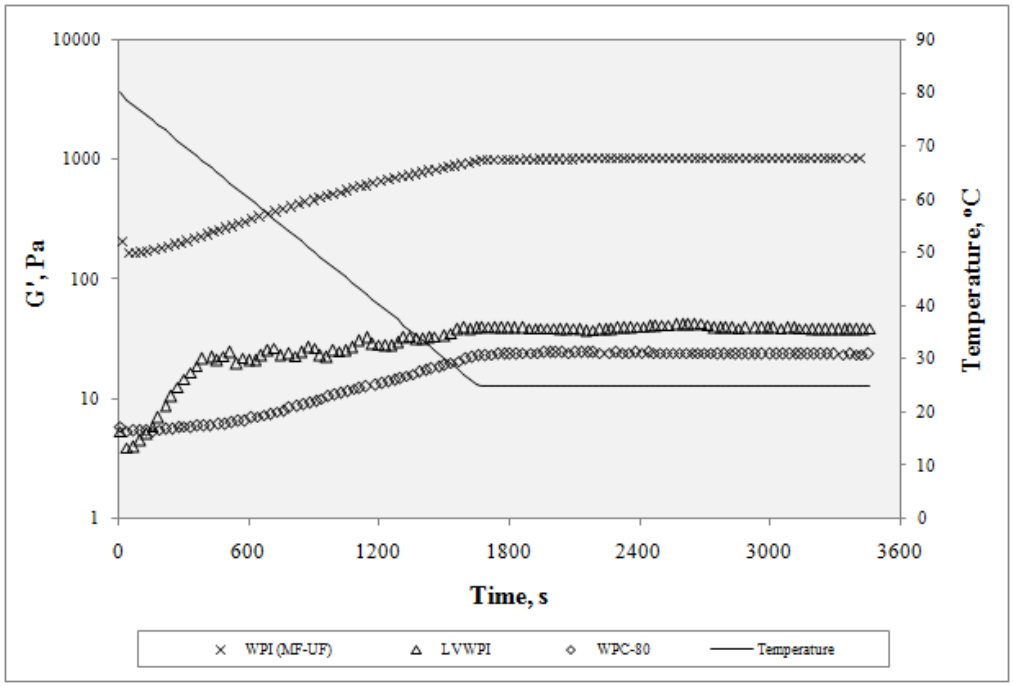

Figure 2.4. Rheological behavior of 8% (w/w) protein solutions at pH 6.0 in small-strain test during cooling to 25°C after 30-minute heating at 80 °C.

heating time of LVWPI was extended from 30 to 180 minutes, its G' value approached but did not equal that of MF-UF WPI (Figure 2.5). Although longer heating time leads to more extensive formation of high-molecular weight WP aggregates that form the primary spatial structure of the gel (Mleko & Foegeding, 2000), an increase in G' for particulate gels requires not only greater amount of protein aggregates at the gel

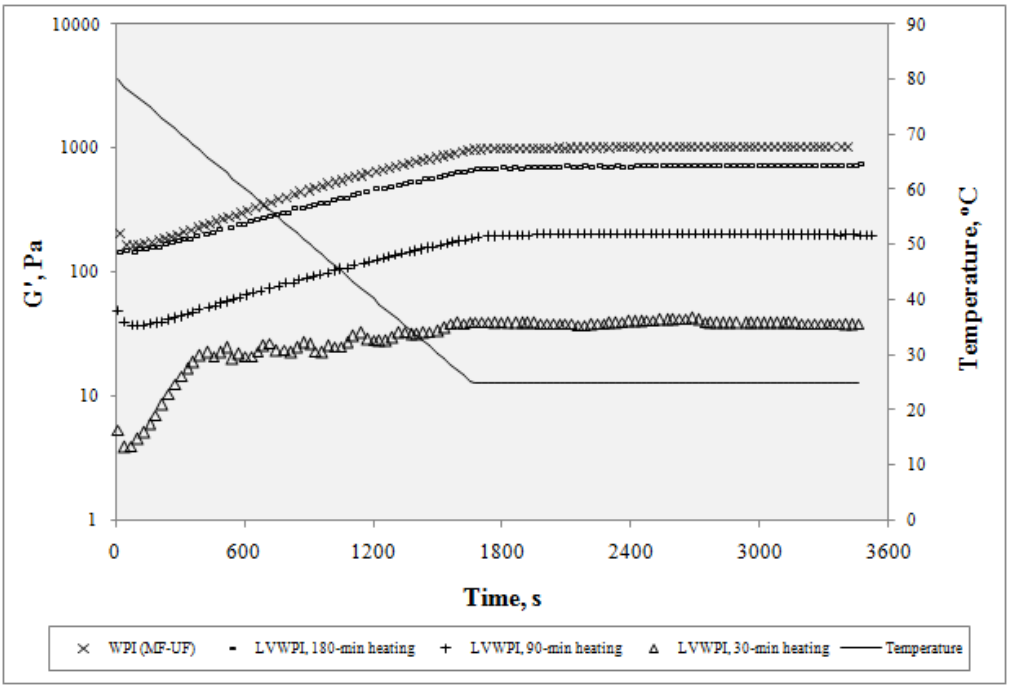

Figure 2.5. Rheological behavior of 8% (w/w) protein LVWPI solution in small-strain test during cooling to 25°C after heating at 80 °C for 30, 90 and 180 minutes compared with 8% (w/w) protein WPI solution heated at 80 °C for 30 minutes.

point but also stronger inter-particle forces among the aggregates, which can be promoted by sufficient amount of salt (Paulsson & Dejmek, 1990; Stading & Hermansson, 1990; Tang *et al*., 1993; Boye *et al*., 1997; Verheul & Roefs, 1998). Since the LVWPI and MF-UF WPI solutions had equal protein contents and that their total mineral contents were similar, the higher G' exhibited by the MF-UF WPI gel, therefore, may have been due partly to the difference in the initial amount of native

proteins content, but more so to the difference in their mineral profiles as suggested by the DSC results.

Caussin *et al*. (2003) showed that particulate WP gels are formed in the presence of about 100 mM of monovalent cations, such as Na^{+}, or about 10 mM divalent ions, such as Ca^{2+}. The addition of $CaCl_2$ to LVWPI solution increased its G' on cooling, after heating at 80 °C for 30 minutes (Figure 2.6). Interestingly, the G' of

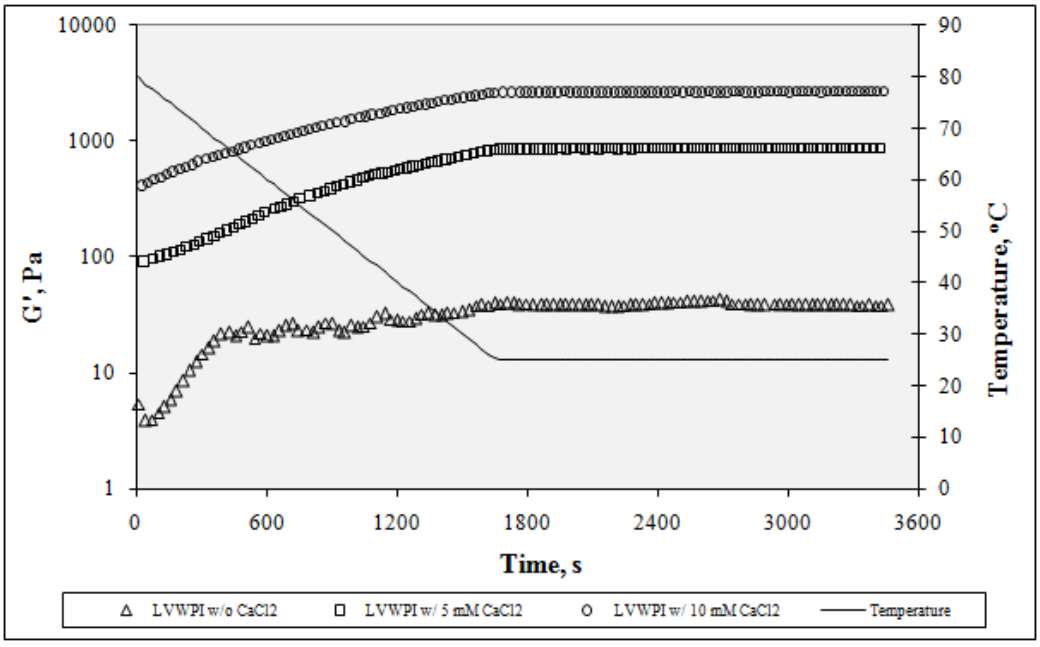

Figure 2.6. Rheological behavior of 8% (w/w) protein LVWPI solution containing $CaCl_2$ in small-strain test during cooling to 25°C after heating at 80 °C for 30 minutes compared with 8% (w/w) protein WPI solution without $CaCl_2$ heated at 80 °C for 30 minutes.

LVWPI gel made by heating 8% (w/w) protein solution with 5 mM $CaCl_2$ at 80 °C for 30 minutes increased its G' to a value equal to that of the MF-UF WPI gel, which was not added with $CaCl_2$ and heated to the same heating conditions (Figure 2.7). More interestingly, when 10 mM $CaCl_2$ was added to LVWPI, the G' of the gel formed was equal to that of the IE WPI gel, which was also prepared with 10 mM $CaCl_2$ and heat-

treated at the same conditions (Figure 2.7). Consistent with previously reported findings, these observations indicate that the addition of $CaCl_2$ to LVWPI aided in increasing the rate of aggregates formation, which increased the rate of native protein denaturation and incorporated more aggregates in the gel network that eventually led to an increase in G' (Verheul & Roefs, 1998).

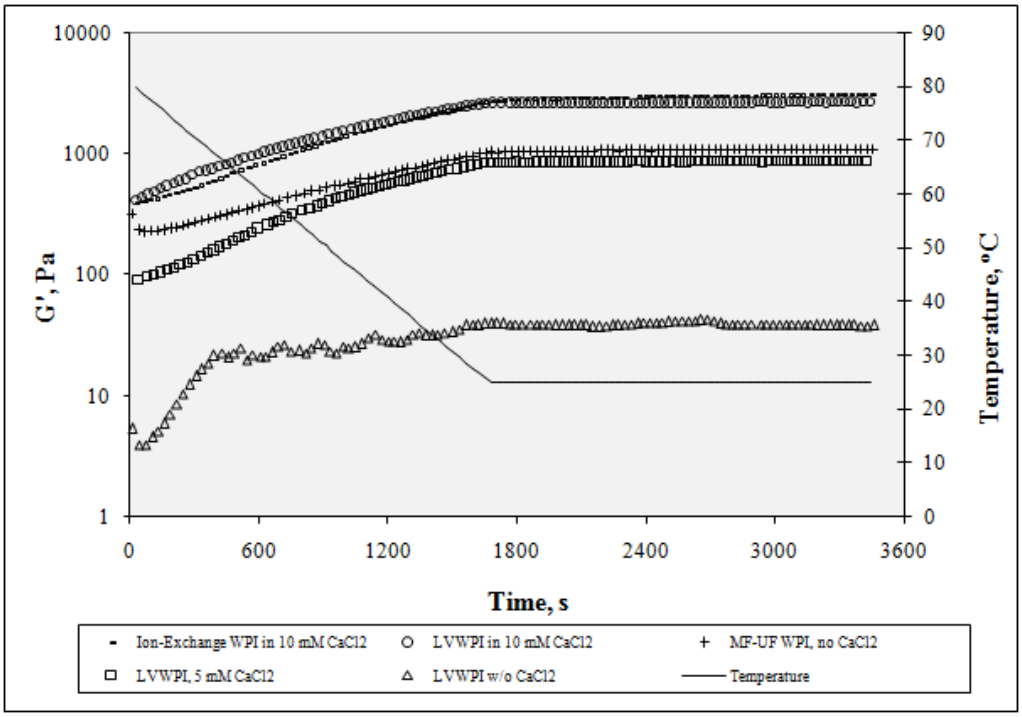

Figure 2.7. Rheological behavior of 8% (w/w) protein LVWPI solution containing 10 mM $CaCl_2$ in small-strain test during cooling to 25°C after heating at 80 °C for 30 minutes compared with 8% (w/w) protein ion-exchange WPI solution with 10 mM $CaCl_2$ and MF-UF WPI solution without $CaCl_2$.

The varying amounts of $CaCl_2$ necessary for LVWPI gel's G' to equal those of MF-UF WPI and IE WPI indicate that with mineral modulation, LVWPI can mimic the gelling behavior, and therefore the texture development capabilities of both commercial products manufactured from cheese whey and concentrated by different methods. Confocal images of the gels formed from these three products without the

addition of $CaCl_2$ confirmed this (Figure 2.8). At the same heating temperature and time, and equal protein contents, the MF-UF WPI solution formed opaque particulate gel that the confocal image showed to be made of large aggregates (Figure 2.8a). The IE WPI solution formed transparent gel with structure made up of fine strands (Figure 2.8b). The LVWPI gel, however, was translucent and the confocal image (Figure 2.8c) showed strands that were thicker than those found in the IE WPI gel and smaller aggregates than those found in the MF-UF WPI gel. Since the protein contents of the gels were equal and that their total mineral contents were numerically similar, it is reasonable to believe that the difference in their texture was brought about by the difference in their mineral profiles (Table 2.3). The IE WPI contained the lowest amount of Ca, which is known to aid in gel network formation by crosslinking

Table 2.3. Mineral profile of commercial WPI and LVWPI.

Element	LVWPI[a]	Ion-Exchange WPI[b,c]	MF-UF WPI[b,d]
Ca	2.1	1.3	5.3
Na	1.3	6.0	1.8
K	0.8	0.6	4.1
Fe	0.006	0.005	0.77
P	7.2	0.75	2.5
Mg	0.034	0.25	1.3

[a] From "virgin whey" diafiltered using phosphate buffer.
[b] From sweet whey.
[c] From Davisco Foods Davisco Foods International (MN, USA)
[d] From Glanbia Nutritionals (Monroe, WI, USA)

negatively charged unfolded protein molecules (Kinsella, 1984) while the MF-UF WPI contained more than twice as much Ca as LVWPI. Also, LVWPI contained the least amount of Na, which is also known to aid in WP gel network formation, although

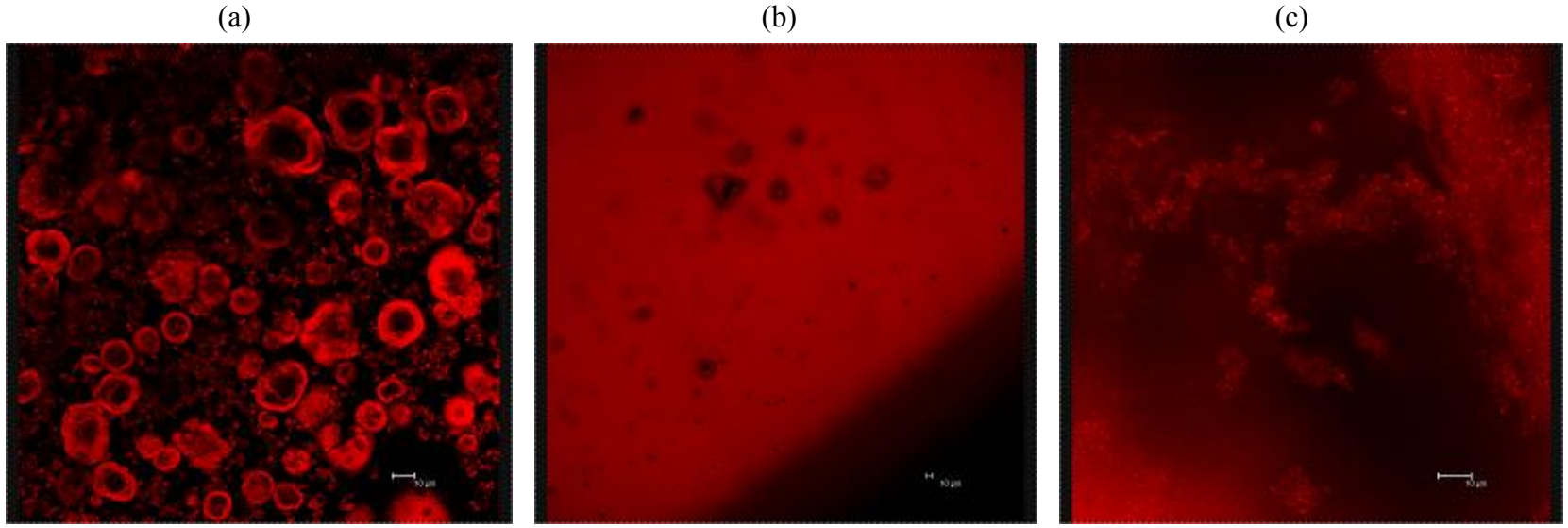

Figure 2.8. Confocal images of heat-induced gels formed from 10% (w/w) protein solutions at pH 6 using (a) MF-UF WPI, (b) ion-exchange WPI, and (c) LVWPI at 80 °C for 10 minutes. The bars represent 10 μm.

to a lesser extent as Ca. Being rich in native WP and having a mineral profile that differs from those of commercial WPI products render LVWPI unique gelling behavior that may prove advantageous in texture development for various food applications.

2.5. CONCLUSION

The previously reported unique physicochemical properties of LVWPI have been shown to translate to a unique functional behavior. The high level of native proteins contained in LVWPI and its mineral profile rendered thermal stability to the product, as shown by its higher onset of denaturation and enthalpy of denaturation compared with commercial MF-UF WPI. This further enabled controlled heat-induced aggregation and gelation through mineral modulation that allowed fine tuned texture development potentials that spanned those of commercial MF-UF WPI and IE WPI. For the first time, it has been documented that membrane processed WP may have such potentials.

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CHAPTER 3

Modeling of batch ultrafiltration for the concentration of virgin whey

3.1. ABSTRACT

A mathematical model that describes flux decline in the ultrafltration (UF) of virgin whey (VW) was derived from one of the flux models used in the UF of cheese whey. The present model was based on the hypothesis that since VW is free of cheesemaking remnants, richer in native whey proteins compared with cheese whey, and that its composition is constant, its long-term UF fouling behavior will be consistent at a given set of operating parameters. Both the short-term flux decline and average flux will depend primarily on the ratio of total UF feed (F) to membrane area (A). The derived equation consisted of two fouling parameters: F/A, which quantifies short-term fouling, and m, which quantifies long-term fouling. Different amounts of VW were concentrated 13 times using polysulfone membrane in spiral wound configuration (10,000 molecular weight cut-off) at 45 °C, 338 kPa transmembrane pressure, and 0.5 m s^{-1} crossflow velocity. Results confirmed that long-term fouling behavior is consistent as shown by a constant m value, while the average flux changes significantly with the F/A value. The present flux equation does not only aid in understanding VW fouling behavior but may also be used as design equation for UF system optimization.

3.2. INTRODUCTION

Although the fractionation of cheese whey into protein-rich (retentate) and lactose-rich (permeate) streams is one of the more successful industrial applications of ultrafiltration (UF), the utility of cross-flow UF for whey processing continues to be a challenge, mainly because of the decrease in permeate flux during operation [1, 2, 3]. UF permeate flux is limited by a few factors, the most significant of which are concentration polarization (CP) and membrane fouling [4, 5, 6, 7]. CP occurs when a concentration gradient of the retained components is formed on or near the membrane surface while fouling is the largely irreversible deposition of material on the membrane surface or within its pores [8]. Mulvihill and Ennis [9] reported that, due to flux decline, the limit for whey concentration by UF in modern plants is ~24% total solids, with a protein to total solids ratio limit of ~0.72:1.00.

Both CP and fouling can be minimized by the appropriate combination of process parameters such as transmembrane pressure (TMP), feed velocity or recirculation rate, temperature, and the physicochemical conditions of whey [10,11, 12]. The membrane material and the structural conformation of the whey proteins (WP) were also found to adversely affect permeate flux through membrane-protein interactions [6, 13, 14].

The efficiency of UF system in whey concentration, therefore, starts from a well-designed process. Mathematical modeling is an important step in the development of UF processes for flux behavior prediction, design and optimization purposes [15, 16].

3.2.1. Concentration of whey proteins by ultrafiltration

Cheese whey contains cheesemaking remnants, fats and spores. Varied cheese manufacturing practices result in compositional variability of whey as well as

fractional denaturation of WP [17, 18, 19, 20, 21, 22, 23, 24]. During UF processing, the partial denaturation of WP in cheese whey aggravate protein-protein and membrane-protein interactions that result in membrane fouling [2, 5, 11, 12, 25, 26]. Protein-protein interactions induce aggregation in solution and/or surfaces pre-adsorbed with proteins, while protein-membrane interactions may lead to pore narrowing and plugging, and cake deposition [12]. The compositional variability of cheese whey gives rise to differences in the nature of the deposit on the membrane [13]. This impedes the accurate prediction of UF flux behavior using a particular flux model, which in turn impedes process optimization.

In the vatless manufacture of cheese, virgin whey (VW) is harvested as permeate from the microfiltration (MF) of slightly acidified (pH 6.0) skim milk to a concentration factor (CF) of 8 before cheesemaking [27]. Therefore, VW does not contain cheesemaking remnants, fats nor spores [27]. Not subjected to extreme physicochemical conditions changes during cheesemaking and pretreatment prior to concentration process, VW proteins are in their native conformation. Therefore, both protein-protein and membrane-protein interactions during UF process can be minimized, leading to minimal occurrence of fouling. In separate experiments, Brandsma and Rizvi [28], Punidadas and Rizvi [29], Solanki and Rizvi [30] and Ardisson-Korat and Rizvi [27] showed that the VW's composition is consistent. Therefore, an appropriate mathematical model will allow the understanding and effective prediction of fouling behavior of VW during UF processing. The resulting model can be used for optimized process design.

3.2.2 Hypothesis

Over the past three decades, attempts have been made to predict flux behavior during UF of cheese whey using either protein model systems, which are usually pure

proteins or binary systems, or real whey systems. Assuming that the amount of flux decline is a function of cumulative permeate volume, Merin and Cheryan [13] suggested that initial flux decline in the UF of whey be simply defined by

$$J = J_o V^{-b} \tag{3.1}$$

where J = instantaneous flux at any time, t, V = accumulated volume of permeate, J_o = initial flux at t = 0, which is an indication of the resistance to solvent transport by the membrane as well as the concentration polarization layer formed on the membrane by the proteins, and b = indicator of the rate of fouling during long-term operation, or the true fouling effects due to specific membrane-solute interactions.

Using individual WP and cottage cheese whey as feed streams, the authors used the model to speculate how each protein influences flux decline and how their interactions in whey under certain physicochemical conditions affect flux decline in polysulfone (PSf) membrane. While their findings provided useful insights that paved the way to considerable number of studies on the UF of whey, they were limited to the initial flux decline in the UF. Although initial flux decline influences the pseudo steady-state permeation, the actual long-term fouling behavior was not verified.

Kuo and Cheryan [10] utilized the same model to investigate long-term fouling of cottage cheese whey on PSf membrane in spiral wound module (SWM). PSf membrane was chosen for its known cost-effectiveness and high tolerance for pH changes during cleaning. The model proved useful in identifying critical process settings but insufficient in providing basic understanding of membrane-solute interactions, which the authors suggested, have the biggest influence on initial flux decline before attaining quasi-steady flux. The model is limited by its inability to identify the point at which CP and gel-layer formation occur on the membrane surface.

Despite this, it is by far, the most utilized model in understanding fouling in the UF of whey.

In the present study, since VW proteins are in their native conformation, it is reasonable to expect that both protein-protein and membrane-protein interactions will be minimal during UF processing. Therefore, the initial solids deposition on the membrane that gives rise to sharp initial flux decline, or short-term flux decline, would be influenced primarily by the ratio of feed (F) to membrane area (A). With the uniform composition of VW and with the minimal protein-protein interactions, the long-term fouling behavior during the UF of VW is hypothesized to be uniform regardless of F/A values. This means that, given a set of operating conditions, b will be constant. Therefore, in the UF of VW, the Merin-Cheryan equation can be re-written so that J is a function of two flux decline parameters, F/A and b. An optimal membrane module design is then determined by F/A for a given concentration factor of the feed and the optimal operation settings are determined by b.

3.3. DEVELOPMENT OF MATHEMATICAL MODEL

The Merin-Cheryan equation can be used to express flux as a function of permeate volume or mass. In the present study, rather than considering permeate volume, permeate mass was considered instead. Therefore, permeate flux as described in the Merin-Cheryan equation was re-defined in terms of permeate mass. By mass balance around the membrane module, the permeate flux can be defined as

$$J = \frac{1}{A}\frac{dV}{dt} \qquad (3.2)$$

When equations (3.1) and (3.2) were combined and integrated, equation (3.3), which was an expression for the mass of permeate was obtained.

$$V = kt^{\frac{1}{b+1}} \tag{3.3}$$

where k is a constant defined as

$$k = \left[J_o A(b+1)\right]^{\frac{1}{b+1}} \tag{3.4}$$

Taking the natural logarithm of both sides of equation (3.3) will yield

$$\ln V = \ln k + \frac{1}{b+1}\ln t \tag{3.3'}$$

Plotting ln V vs. ln t in equation (3.3') will allow the estimation of the parameters b and k. Taking the differential of equation (3.3) and combining with equation (3.2), an expression for flux is obtained,

$$J = \frac{k}{A(b+1)} t^{\frac{-b}{b+1}} \tag{3.5}$$

Following the modeling approach of Tekić *et al*. [15], overall mass balance around the membrane module at any time, t, gives

$$V_R = F - V \tag{3.6}$$

where V_R, F and V are the mass of retentate, feed and permeate, respectively. Protein balance gives

$$-\frac{d(V_R C)}{dt} = JAC(1-R) \tag{3.7}$$

where J is the permeate flux at any time, t, C is the mass concentration of protein, and R is the rejection coefficient, which is defined as

$$R = \frac{C - C_p}{C_o} = 1 - \frac{C_p}{C_o} \tag{3.7a}$$

where C, C_p and C_o are the concentration of protein in the retentate, permeate and feed, respectively. Combining equations (3.5), (3.6) and (3.7), and integrating the resulting differential equation gives an expression for the relative quantity of proteins in the retentate and feed as a function of time and R,

$$\frac{V_R C}{F C_o} = \left\{ 1 - \frac{\left[J_o A (b+1)^{\frac{1}{b+1}} \right]}{F} t^{\frac{1}{b+1}} \right\}^{(1-R)} \tag{3.8}$$

Combining equations (3.5), (3.6) and (3.8), an expression for the relative concentration of proteins in the retentate and feed as function of time can be derived as

$$\frac{C}{C_o} = \left[1 - \frac{k}{F} t^{\frac{1}{b+1}} \right]^{-R} \quad \text{or} \quad \frac{C}{C_o} = \left\{ 1 - \frac{\left[J_o A (b+1) \right]^{\frac{1}{b+1}}}{F} t^{\frac{1}{b+1}} \right\}^{-R} \tag{3.9}$$

Equation (3.9) can be modified to estimate process time for a desired value of C/C_o as

$$t = \left\{ \left[1 - \left(\frac{C}{C_o} \right)^{\frac{1}{R}} \right] \left(\frac{F}{k} \right) \right\}^{(b+1)} \tag{3.10}$$

Equations (3.5), (3.8) and (3.9) express J, and the relative quantities and concentrations of proteins in the retentate and feed, respectively, as direct functions of time and indirect function of F/A and b. It has been hypothesized that b is constant for

VW, regardless of F/A value, while the short-term flux decline is governed by F/A. Therefore, only t and F/A will have a greater influence on J values. And, if an equation that explicitly expresses J as direct function of both t and F/A could be derived, a good fit of the data into the equation would verify the hypothesis.

Solute balance around the module expresses protein concentration in the retentate as a function of CF,

$$C = C_o(CF)^R \quad \text{or} \quad \frac{C}{C_o} = (CF)^R \tag{3.11}$$

Solving equations (3.5) and (3.9) simultaneously gives

$$\frac{C}{C_o} = \left[1 - \frac{JA(b+1)t}{F}\right]^{-R} \tag{3.12}$$

Solving equations (3.11) and (3.12) simultaneously to solve for J yields

$$J = \frac{F}{A}\left(\frac{1}{b+1}\right)\left(\frac{1 - \frac{1}{CF}}{t}\right) \tag{3.13}$$

where: $\frac{F}{A}$ = the hypothesized initial fouling parameter

$\frac{1}{b+1}$ = long-term fouling parameter = m

Equation (3.13) indicates that J is a direct function of the hypothesized fouling parameters, F/A and m. If the hypothesis that b is constant in the UF of VW at specified operating conditions can be proven, then m would also be constant. This means that F/A would be the critical UF design parameter for a given CF.

3.4. MATERIALS AND METHODS

3.4.1. Concentration of virgin whey proteins

Step 1. Microfiltration: Virgin whey recovery

The MF system used was a Tetra Alcross™ megaloop-38 (Tetra Pak Inc., Vernon Hills, IL, USA), which consisted of 38 Membralox® ceramic membrane elements (Pall Corporation, Deland, FL, USA) with nominal pore diameter of 0.1 μm. The elements were 1020 mm long giving an effective filtration area of 9.1 m^2. The process, which involved concentration of slightly acidified skim milk to 8x, was described in detail by Ardisson and Rizvi [27]. The MF permeate, which was the VW, was collected and held in a jacketed stainless steel vat, gently stirred at 45 °C before using as feed stream to the UF system immediately after the MF process. Different amounts of skim milk were used to collect different amounts of VW (950 kg, 1025 kg, 1125 and 1325 kg), which were used as feed in the UF system.

Step 2. Ultrafiltration using spiral wound polysulfone membrane

The VW concentration was carried out using S4-HFK-131-VSV PSf SWM from Koch Membrane Systems, Inc. (Wilmington, MA, USA) with a molecular weight cut-off (MWCO) of 10,000 and an effective filtration area of 5.9 m^2. The SWM was operated at an average pressure drop along the length of the module of 275 kPa, which corresponded to an average cross-flow velocity of 0.5 m s^{-1}. The TMP and temperature were maintained at 338 kPa and 45 °C, respectively. Filtration was continued until a CF of 13 was achieved. The weight of the permeate stream from the SWM was recorded at 10-minute intervals to determine the permeate mass flux. The pH and temperature of the retentate were also monitored at the same time interval. The operating conditions in the MF and UF are summarized in Table 3.1 and the

schematic diagram of the UF system used in the study is shown in Figure 3.1.

Table 3.1. Operating conditions in the microfiltration (MF) and ultrafiltration (UF) systems that were used in the recovery and concentration of virgin whey, respectively.

Parameters	MF Tetra-Alcross®	UF spiral wound module
Feed pH	6.0	6.1
Average temperature, °C	50.2	45.3
P_{inlet}[a], kPa	372	475
P_{outlet}[a], kPa	283	200
Average TMP, kPa	101[b]	338
Average crossflow velocity, m s^{-1}	0.5	0.5
% total solids in feed	9.19[c]	5.31[d]
Final mass concentration factor	~8	~13
Clean water flux, kg h^{-1} m^{-2}	200	62.2
Membrane area, m^2	9.2	5.9

[a] Pressures in the retentate side.
[b] Uniform transmembrane pressure.
[c] GDL-acidified (pH 6) skim milk.
[d] Virgin whey.

3.4.2 Determination of rejection coefficient

Different amounts of VW were used as feed in the SWM at 338 kPa TMP, 45°C and 0.5 m s^{-1} crossflow velocity. Samples of the retentate and permeate were taken at 10-minute intervals until MCF of 13 was reached. The samples were then analyzed of their protein content and R was calculated using the protein concentrations in the permeate and retentate.

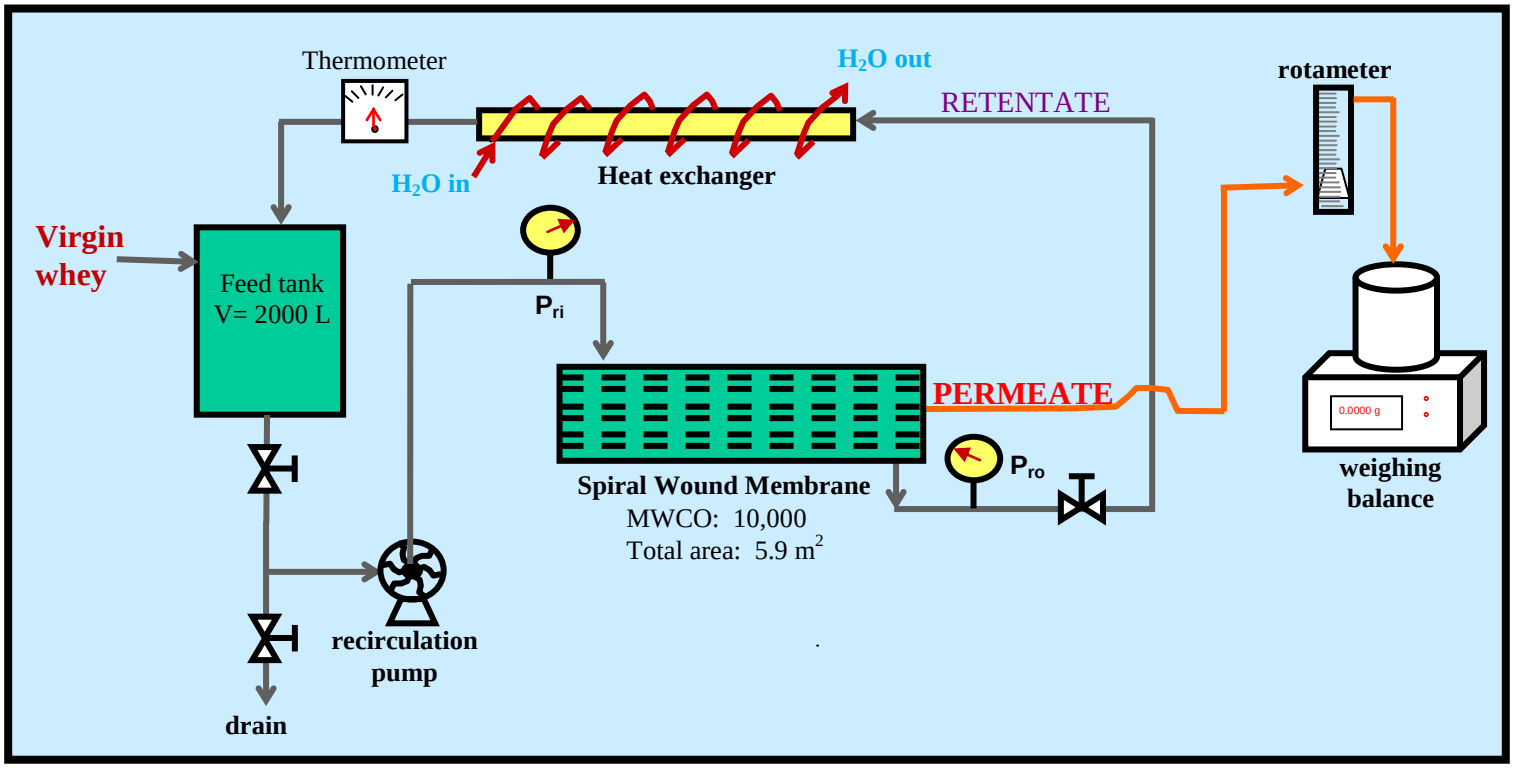

Figure 3.1. Schematic diagram of the ultrafiltration set-up used in the study. P_{ri} is the inlet pressure while P_{ro} is the outlet pressure in the retentate side. The permeate side is open to atmosphere.

3.4.3. Compositional analyses

The composition of UF streams were determined following the AOAC (2000) [31] protocol unless otherwise specified. The % total solids (TS) was determined bydrying in an oven at 100 °C for four hours (AOAC, 2000; 33.2.44, 990.20) [31]. Total nitrogen was determined by Kjeldahl method (AOAC, 2000; 33.2.11, 991.20) [31] and the true protein was obtained after correction for non-protein nitrogen (NPN) (AOAC, 2000; 33.2.12, 991.21) [31] using a protein conversion factor of 6.38. The sample size was adjusted so as to contain similar absolute amount of protein as milk in the recommended amount in the procedure, taking into account the concentration factors at different stages in the process. The true protein fraction was taken as equal to whey protein fraction. All determinations were done in quadruplicates.

3.4.4 Statistical analyses and mathematical modeling

All statistical analyses on experimental data and predicted flux using the developed mathematical model were done using MINITAB® release 14 statistical software (State College, PA, USA).

3.5. RESULTS AND DISCUSSION

3.5.1 Ultrafiltration of virgin whey using spiral wound membrane

The %TS and % true protein (w/w) of VW was 5.31 and 0.32, respectively. When solute concentration in the feed stream is low, such as this case, higher TMP in the UF is necessary to achieve the limiting flux [3]. However, the feed crossflow velocity, which depends on pressure drop along the length of the module, must be minimized in order to lower power consumption. Therefore, a combination of high TMP of 338 kPa and low crossflow velocity of 0.5 m s^{-1} was used in the UF of VW to attain reasonable permeate flux. The TMP of 338 kPa was close to the critical TMP of

335 kPa found by Cheryan and Kuo [1] in concentrating cottage cheese whey using spiral wound PSf membrane. As Brans *et al*. [7] emphasized, concentration of WP must be carried out just above the critical pressure where flux is equal to the limiting flux, to achieve an optimal operation.

As for the choice of membrane configuration, it is advantageous to use a membrane module of large surface area for high-throughput operations. In the present study, the spiral wound configuration was preferred for its high packing density, which significantly increases its surface area [32]. The PSf in spiral wound configuration, which can withstand higher pressures and is able to minimize the occurrence of concentration polarization, also involves the lowest capital and operating cost compared with other configurations [33, 34, 35]. Therefore, the operation parameters used in this study with PSf membrane in spiral wound configuration may also prove cost-effective [1, 36, 5, 7].

3.5.2 Flux decline and fouling behavior of virgin whey

The flux curves in the SWM (Figure 3.2), at any F or F/A value, show three distinct segments as usually observed in the UF of cheese whey: (1) the region of rapid flux decline, which indicated occurrence of concentration polarization and rapid reversible fouling, (2) the pseudo-steady state region where the rate of particle deposition and the rate of particle removal due to surface shear forces are almost equal as made apparent by a flux plateau, and (3) the departure from flux plateau to further decline, which is usually attributed to pore plugging. Starting from a clean water flux of 62.2 kg hr^{-1} m^{-2}, the average permeate mass flux in the SWM was 42.9 kg hr^{-1} m^{-2} during the first 10 minutes of operation for F = 1025 kg. As expected, the average mass flux varied with the feed quantity used (Table 3.2). Over the next 30 minutes, the permeate flux continued to decline with time and plateaued to its pseudo steady-

state value until a CF of about 8. The flux then declined further as the CF reached 13 and the retentate TS reached about 3% (w/w). Above this concentration, Kuo and Cheryan [10], and Nilsson [37] found considerable drop in permeate flux for cheese whey.

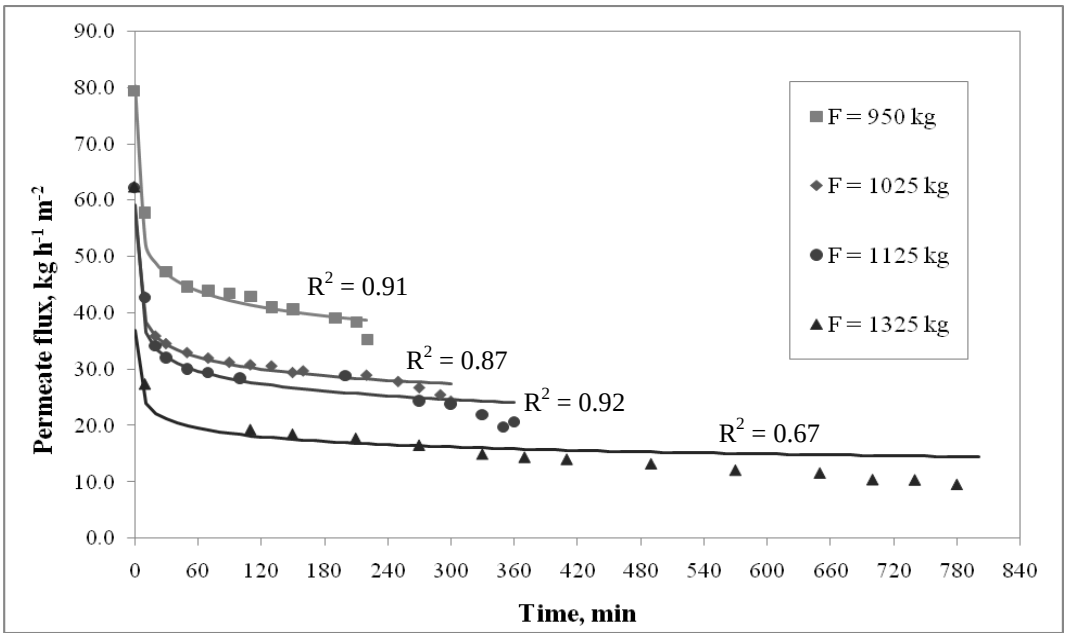

Figure 3.2. Experimental flux data and flux values predicted by the Merin-Cheryan equation in the ultrafiltration of virgin whey using polysulfone membrane (10 kDa molecular weight cut-off) in spiral wound configuration at 45 °C and 338 kPa transmembrane pressure. The curves are model predictions and the points are experimental data.

The pseudo steady-state flux or flux plateau decreased with increase in F/A ratio (Table 3.2). For the lowest feed quantity used in the present study, F = 950 kg, which corresponded to F/A value of 161 kg feed per m^2 membrane area, the SWM permeate initial flux, taken during the first 10 minutes of UF was about 58 kg h^{-1} m^{-2} (Figure 3.2). This observed initial flux was more than twice as high as that reported by Merin and Cheryan [13] of about 20.6 kg h^{-1} m^{-2} in the UF of dialyzed cottage cheese whey at pH 4.6. The previous study was carried out at 174 kPa TMP and 50 ºC

for a much lower approximate F/A value of 34 kg feed per m^2 membrane area using flat sheet PSf membrane (10 kDa MWCO) in a stirred-tank set-up. The much lower F/A value should have resulted to a higher initial flux in the previous study since there are more protein adsorption sites that should take longer to saturate in a larger

Table 3.2. Variation of permeate flux with feed quantity in the ultrafiltration of virgin whey using spiral wound polysulfone membrane at pH 6.1, 45 °C and TMP of 338 kPa up to a concentration factor of 13.

Feed, kg	F/A, kg feed per m^2 membrane area	Average initial flux[a], kg m^{-2} h^{-1}	Average flux[b], kg m^{-2} h^{-1}	Flux plateau, kg m^{-2} h^{-1}	Processing time, min
950	161	51.8	41.7	40.6	240
1025	174	37.8	30.1	30.0	330
1125	191	36.3	25.9	25.2	395
1325	225	23.8	14.8	14.0	885

[a] Average permeate mass flux during the first 30 minutes of operation.
[b] Average permeate mass flux all throughout the operation.

membrane area. Aside from the difference in TMP and the mode of operation, the difference in the physicochemical characteristics, such as pH, of VW and the cottage cheese whey dialysate may have been a major factor in the large difference between the observed initial flux values in the present study and that of Merin and Cheryan [13]. It is likely that the hydrophobic membrane-protein interaction, which is more severe when the proteins are denatured, was greater in the previous study leading to lower flux [5, 25]. At the VW pH of 6, the whey proteins and the PSf membrane have negative charges, which may have promoted repulsion that led to a lower extent of membrane-protein interactions.

The results of the present study also show about six times improvement in the flux plateau value compared with what Kuo and Cheryan [10] observed in the UF of pH-adjusted and prefiltered cottage cheese whey (pH 7) at 50 ºC and a similar TMP of 310 kPa using spiral wound PSf membrane (20 kDa MWCO). About 30% increase in the flux plateau was also evident in the present study compared with the value reported by Rektor and Vatai [38] in the UF of mozzarella cheese whey using spiral wound PSf membrane (10 kDa MWCO). The flux values with respect to protein concentration in the present work are comparable with those presented by Kessler [39] on UF of whey at similar TMP of 334 kPa, lower temperature of 35 ºC but at a much higher tangential velocity of 2.5 m s^{-1} using a tubular module. Although data from literature allow comparisons of membrane performances in terms of flux, they do not assess the efficiency of the UF system design because the F/A values are not specified. Since protein fouling of membranes initially occurs by physical adsorption in a monolayer [5], the F to A ratio is an important parameter that may have considerable influence on flux decline. Therefore, comparisons between UF systems become more meaningful if F/A values are also specified in addition to the operating parameters.

3.5.3 Mathematical modeling

Using the Merin-Cheryan equation, for F/A between 161 and 225 kg of VW per m^2 membrane area, changes in the b values were insignificant ($p \leq 0.05$), with an average value of 0.12 (Table 3.3) in the UF of VW at 45 °C, 338 kPa TMP and 0.5 m s^{-1} crossflow velocity. This confirms that, for as long as the operating conditions that define the UF system hydrodynamics remain the same, the long-term fouling behavior of VW does not change significantly with F/A values between 161 and 225 kg VW per m^2 membrane area. Results also show that b varies significantly with TMP when F/A was constant ($p \leq 0.05$). This observation is consistent with previous findings that

membrane fouling is influenced by both the hydrodynamics of the filtration process and the surface interactions between the membrane and the foulants [25]. Since after initial protein monolayer adsorption, protein build-up on the membrane takes place via intermolecular disulfide bonding and hydrophobic interactions, the composition of whey and the structural conformation of the proteins influence long-term fouling [5].

Table 3.3. Merin-Cheryan fouling model parameters at different feed quantities and transmembrane pressures in the ultrafiltration of VW at pH 6.1 and 45 °C.

Feed, kg	F/A, kg feed per m^2 membrane area	Transmembrane pressure, kPa	J_o, kg min^{-1}	B	$R^{2\,a}$
950	161	338	7.90	0.11	0.99
1025	174	338	5.66	0.11	0.99
1125	191	338	5.82	0.13	0.99
1325	225	338	3.64	0.13	0.99
1025	174	290	5.21	0.16	0.99
1025	174	210	3.26	0.21	0.99

[a] Goodness of fit.

The constant composition of VW and its richness in native proteins allowed consistent behavior during UF processing, which was manifested by the constant value of b regardless of F/A value. The increasing value of b with decreasing TMP at 0.5 m s^{-1} crossflow velocity suggests more massive long-term fouling at lower TMP values. This indicates that 338 kPa is the appropriate TMP for VW processing.

At the same operating temperature and TMP, J_o changes significantly with F and/or F/A ($p \leq 0.05$), which indicates that F/A influences short-term flux decline (Table 3.2). Also, the average flux decreased as F/A increased (Table 3.3), which

indicates that the average flux is also a strong function of F/A. These observations show that the desired average flux can be attained by using the appropriate F/A value.

For F/A values of 161, 174 and 191 kg VW per m^{-2} membrane area, the flux values predicted by equation (3.5) show good agreement with observed flux values (Figure 3.2), with goodness-of-fit (R^2) of 0.91, 0.87 and 0.92, respectively. This indicates the appropriateness of the Merin-Cheryan equation to predict the flux decline pattern of VW. However, when F/A was 225 kg VW per m^2 membrane area, the R^2 value was lower at 0.67, indicating that the satisfactory use of the Merin-Cheryan equation is limited at F/A values lower than 225 kg VW per m^2 membrane area. Evidently, at higher F/A values, the influence of F/A on fouling, which is not directly considered in the equation, become more pronounced.

Pilot-scale test runs indicate that the R value in the UF of VW using 10 kDa MWCO PSf was 0.98. Using this value and equation (3.9), the relative concentration of proteins in the SWM retentate to the initial concentration in the feed, or C/C_o, can be predicted. Results show that the estimated values are in good agreement with experimental data (Figure 3.3). For F/A values of 161, 174 and 191 kg VW per m^2 membrane area, R^2 values were 0.98, 0.96 and 0.96, respectively. Therefore, using the modified Merin-Cheryan equation, the concentration of protein in the SWM retentate at any time can be estimated. However, equation (3.9) uses the parameter k or J_o, which are both functions of F/A. Therefore, expressing C/C_o as a direct function of F/A, and using such expression to solve for J will be a more convenient approach in predicting permeate flux.

Having established that the average permeate flux is a strong function of F/A and the validity of the modified Merin-Cheryan equation in estimating flux history in the UF of VW as shown by both Figures 3.2 and 3.3, flux can be expressed as an explicit function of F/A as shown in equation (3.13). This equation has two fouling

parameters: F/A, which estimates short-term flux decline, and m, which is based on the Merin-Cheryan long-term fouling parameter, b. Since b is a constant value of 0.12 explicit function of F/A as shown in equation (3.13). This equation has two fouling parameters: F/A, which estimates short-term flux decline, and m, which is based on the Merin-Cheryan long-term fouling parameter, b. Since b is a constant value of 0.12

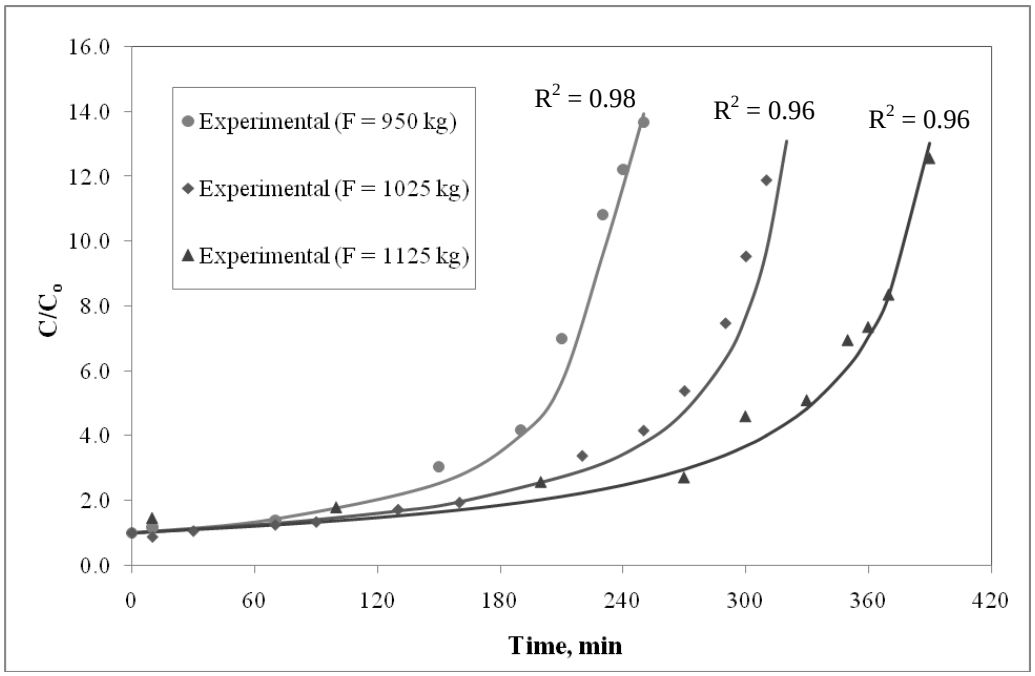

Figure 3.3. Experimental and predicted relative protein concentrations in spiral wound membrane retentate in the ultrafiltration of virgin whey (pH 6.1) to concentration factor of 13 at 45 °C and 338 kPa transmembrane pressure. The curves are model predictions and the points are experimental data.

in the UF of VW at 45 °C and TMP of 338 kPa then, at these conditions, m assumes a constant value of 0.89. Also, CF and t were put together as a single independent variable in (1-(1/CF))/t. The calculated flux values using the derived model show good agreement with experimental values, which means that the model can be used to estimate permeate flux in the UF of VW (Figure 3.4). The R^2 values are 0.86, 0.86 and 0.93 for F/A values of 161, 174 and 191, respectively. For the higher F/A value of

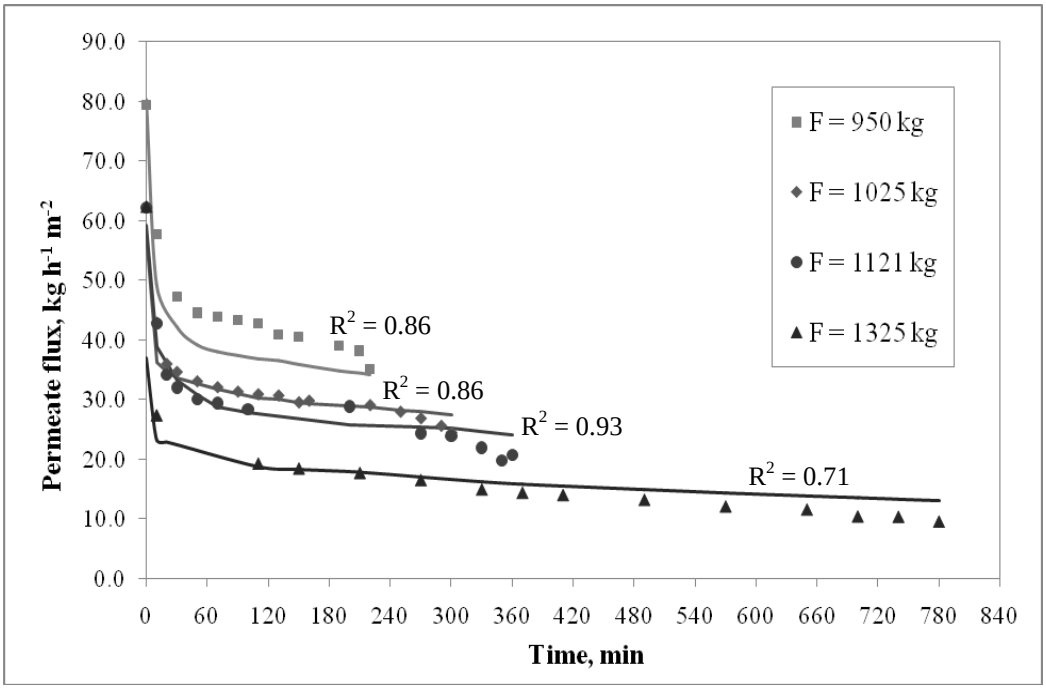

Figure 3.4. Experimental flux data and flux values predicted by the developed model with fouling parameters F/A and m, in the ultrafiltration of virgin whey (pH 6.1) using polysulfone membrane (10 kDa molecular weight cut-off) in spiral wound configuration at 45 °C and 338 kPa transmembrane pressure. The curves are model predictions and the points are experimental data.

225, R^2 was 0.71, which is slightly higher than that found for equation 3.5. These findings confirm the hypothesis that a two-parameter flux equation, in terms of F/A and m, describes the UF of VW. Equation 3.13 can then be used as a design equation in determining the optimal F/A value for a UF system.

3.6. CONCLUSION

The two-parameter model developed in this study showed satisfactory agreement with actual flux data in the UF of VW using spiral wound PSf membrane at 45 °C and 338 kPa TMP. The model showed two things in the UF of VW: (1) The long-term fouling is governed by the process hydrodynamics and its occurrence is consistent regardless of the amount of feed in a given membrane area for F/A value up

to 225 kg VW per m^2 membrane area. This is due to the constant composition of VW and the WP being in their native form. (2) The critical design parameter in a UF system for VW processing is F/A, which influences both the initial and average flux values. This indicates that a well-designed UF system involves an optimal F/A value. The flux model developed can be a practical design equation for use in the industry in the large scale processing of VW.

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CHAPTER 4

Process analyses of batch ultrafiltration for the concentration of virgin whey

4.1. ABSTRACT

A two-stage ultrafiltration (UF) with diafiltration process was designed to produce liquid virgin whey protein isolate (LVWPI), a novel ingredient rich in native whey proteins (WP). Virgin whey (VW), the permeate from the microfiltration of slightly acidified skim milk (pH 6.0), was the feed stream. Process parameters and membrane configurations were chosen based on literature values for UF of cheese whey. Results indicated that compared to flux values reported in the UF of cheese whey, there was an improvement in VW flux. This was attributed mainly to VW's richness in native WP which may have minimized both protein-protein and membrane-protein interactions, the major factors that contribute to flux reduction. Minimum process energy requirement and membrane costs were estimated, using a flux model previously derived, which allowed the determination of optimum UF feed (F) to membrane area (A) ratio in the UF of VW.

4.2. INTRODUCTION

Membrane technology has evolved into a major unit operation in the dairy industry today as it provides unique opportunities to accomplish both the fractionation and concentration of components in dairy systems. In whey processing, ultrafiltration (UF) is important because of its advantages over conventional separation and concentration methods in processing whey to the desired level of soluble native proteins, lactose and minerals (Barba *et al*, 2001). UF allows the production of whey proteins (WP) concentrate without phase change, retaining the desirable physicochemical characteristics and functional properties of WP (Brans *et al*., 2004). Compared to most separation technologies, UF is also less energy intensive and does not require chemical treatment during processing (Krishna Kumar *et al*., 2004).

The vatless manufacture of cheese is one novel process where membrane technology plays a major role. In this process, virgin whey (VW) is harvested from the microfiltration (MF) of slightly acidified (pH 6.0) skim milk to a mass concentration factor (MCF) of 8 before cheesemaking. This gives VW an advantage over cheese whey in that it does not contain cheesemaking remnants, fats nor spores (Ardisson-Korat & Rizvi, 2004). In separate studies, Brandsma and Rizvi (2001), Punidadas and Rizvi (2001), Solanki and Rizvi (2001), and Ardisson-Korat and Rizvi (2004) showed that the VW's composition is consistent. Not subjected to extreme changes in physicochemical conditions during cheesemaking and often complex pretreatment prior to concentration process, the WP in VW are in their native form, and therefore of excellent functional properties (Marcelo & Rizvi, 2008). The minimal protein-protein interactions among the native WP and their low resistance to viscous flow enables concentration to a high protein content by UF alone to produce liquid virgin whey protein isolate (LVWPI), a native protein-rich novel ingredient of advantageous physicochemical properties over commercial whey protein isolates

(WPI) manufactured from cheese whey by MF-UF (Marcelo & Rizvi, 2008).

The goal of this study was to conduct process analysis and energy requirements estimation in LVWPI production using a pilot-scale UF system. A flux model developed previously, which shows that the ratio of feed quantity (F) to membrane area (A) is a critical design parameter in the UF of VW, was used to determine the optimal combination of F and A that will give minimal energy consumption and membrane cost. Process settings in the UF of cheese whey given in the literature were considered, verified as optimal, and utilized as process settings in the present study.

4.3. PROCESS DESIGN

4.3.1 Membrane and physicochemical conditions selection in the ultrafiltration of virgin whey

The polymeric polysulfone (PSf) membrane remains to be the most widely used membranes in whey UF primarily because of its low cost, good thermal stability and mechanical properties (Qin *et al*., 2003; Brans *et al*., 2004). It is commonly believed that compared with ceramic membranes and hydrophilic polymeric membranes, the hydrophobic PSf membrane gives lower fluxes and more severe fouling (Marshall *et al*., 1993). However, Doyen *et al*. (1996) showed that in UF of whey, practically the same flux/concentration factor and whey permeability coefficient is obtained using PSf and ceramic membranes. The main challenge of using PSf is the minimization of the hydrophobic and electrostatic interactions between the membrane and the WP that usually lead to massive fouling (Marshall *et al*., 1993; Palecek & Zydney, 1994; Yoo *et al*., 2003). Since hydrophobic interactions become severe if the proteins were denatured (Daufin *et al*., 2001), flux decline is likely minimized if the feed stream was rich with native proteins, such as VW.

Opposite charges on the WP and the membrane induce protein-membrane electrostatic attractions that initiate protein adsorption on the membrane surface (Hanemaaijer *et al*., 1989; Marshall *et al*., 1993; Koehler *et al*., 2000). This may result in undesirable denaturation and aggregation of the adsorbed proteins, especially at high-shear operations (Sheldon *et al*., 1991; van Reis *et al*., 1997). Since PSf has a negative charge from pH 2 to 10 (Marshall *et al*., 1993; Doyen *et al*., 1996), operating above the average isoelectric point of WP (about pH 5.1) may induce electrostatic repulsion between the proteins and the membrane, thereby limiting the occurrence of protein-membrane interactions. At pH 6, β-Lg is known to be in its most compact native configuration (Timasheff *et al*., 1966; Casal *et al.* 1988; Taulier & Chalikian, 2001) while α-La, which was found to have the greatest gel-forming tendency in UF PSf membranes that causes immediate loss of initial flux (Merin & Cheryan, 1980; Hanemaaijer *et al*., 1989), is monomeric and has very little tendency to undergo aggregation (Klostergaard & Pasternak, 1957; Kronman & Andreotti, 1964; Griko, 1999). Therefore, maintaining the pH at 6.0 may contribute to a reasonable permeate flux.

Although the WP themselves are the major foulants, calcium and phosphates have been directly implicated with membrane fouling as possible catalysts or bridging agents between the proteins and the membrane or the proteins themselves, and the formation of insoluble calcium salts (Muller & Harper, 1979; Merin & Cheryan, 1980; Hanemaaijer *et al*., 1989; Labbé *et al*., 1990; Marshall *et al*., 1993). Rao (2002) observed that for both sweet whey and acid whey, flux was controlled by fouling through gradual adsorption of WP to the membrane surface and pore plugging by precipitated calcium phosphate. Hanemaaijer *et al*. (1989) found that the UF membrane characteristics do not influence the deposition of calcium phosphate as strongly as pH and temperature. They observed membrane rejection of Ca at higher

pH and temperature because its solubility decreases at these conditions (Maubois, 1980). Their findings were consistent with those of Kuo and Cheryan (1983). Labbé *et al*. (1990) found that phosphates, either calcium phosphate, apatite and hydroxyapatite at pH 6.9, or sodium hydrogen phosphate at pH 5.6, were the main mineral foulants in the UF of raw and clarified whey using ceramic membranes. The same authors suggested the formation and adsorption of calcium-phosphate-protein complexes on the membrane surface at high pH, which explains the gelatin-like and firmly compacted fouling layer at high pH and the loose fouling layer at low pH observed by Kuo and Cheryan (1983). Using sweet whey, Hanemaaijer *et al*.'s (1989) data showed that calcium permeates satisfactorily through an acrylic copolymer membrane, 30 kDa-molecular weight cut-off (MWCO), at pH 6.0 and 45 ºC at a permeate to initial feed calcium content ratio of about 0.9 after two hours of process time. Marshall and Daufin (1995) pointed out that around pH 6, calcium changes to a more soluble form and that phosphate is in the soluble sodium hydrogen phosphate. Therefore, it is plausible to effectively control flux decline in PSf membrane by setting temperature and pH at 45 °C and 6.0, respectively.

4.3.2 Membrane Configuration

Although flux decline may be minimized through appropriate process variables, energy consumption is a function of these variables (Cheryan & Kuo, 1984). While TMP-flux relationships (for pressure-controlled systems) and fluid velocity-flux relationships (for mass-transfer controlled systems) are relatively independent of module design (Cheryan & Kuo, 1984; Marshall *et al*., 1993; Doyen *et al*., 1996), the pressure drop-fluid velocity relationship, and thus energy consumption, is a characteristic of a specific module design (Cheryan & Kuo, 1984). PSf membranes are usually in the spiral wound (SW) or hollow fiber (HF) configurations. The

available HF modules in the industry are limited by their low TMP ratings. The SW modules, which can withstand higher pressures and are able to minimize the occurrence of concentration polarization, involve the lowest in capital and operating cost compared with other configurations (Mulder, 1991; Cheryan, 1998; Krishna Kumar *et al*., 2004). Their high packing density, which significantly increases their surface area, is advantageous for high-throughput operations (Yee *et al*. 2007).

Depending on process objective, UF can be carried out below, above or at the critical TMP at which the flux ceases to increase linearly with increase in TMP, and therefore, referred to as the "limiting flux" (van Reis *et al*., 1997; Brans *et al*., 2004). When the feed solute concentration is low, higher TMP is required to achieve the limiting flux (Carić *et al*., 2000). Therefore, using SW to concentrate large volume of low solute-concentration feed, such as whey, is practical. On the other hand, the HF configuration has the advantage of giving higher flux than the SW due to higher shear rates developed in the module for the same pressure drop (Cheryan & Kuo, 1984; Cheryan, 1998). This is of advantage for high solute-concentration systems, such as pre-concentrated whey, where permeation is more likely to be mass-transfer controlled and a high crossflow velocity is needed to maintain reasonable flux (Cheryan & Kuo, 1984; Brans *et al*., 2004). Therefore, it is practical to concentrate whey using a two-stage UF with SW in the first-stage followed by HF in the second stage.

4.3.3 Optimal process variables settings

Using tubular ceramic membrane, Aimar *et al*. (1988) showed that in the UF of sweet whey (pH 6.3), there was no considerable difference in flux plateau values at crossflow velocities from 1.8 to 4.0 m s^{-1} at 50 °C and TMP of 300 kPa. This indicates that the critical TMP in UF of whey is around 300 kPa, although the absolute flux plateau values might be affected by pH and the membrane material and

configuration. Kuo and Cheryan (1983) found that the critical TMP in the UF of pre-filtered cottage cheese whey, acidified to pH 3, at 50 °C using 20 kDa-MWCO PSf membrane in SW configuration, was between 310 and 350 kPa. At higher pressures, these authors pointed out that, even at high flow rates, flux declined rapidly due to extensive fouling and deposit layer compaction, reiterating that higher flow rates are beneficial only at pressures below some critical pressure (Marshall *et al*., 1993; van Reis *et al*., 1997). Brans *et al*. (2004) suggested that concentration of whey should be carried out just above the critical pressure where flux is equal to the limiting flux, to achieve optimal operation. Therefore, in the UF of VW, it seemed that the optimal TMP setting in the SW would be around 330 kPa. Kuo and Cheryan (1983), however, did not find critical TMP for HF because of the limited pressure rating of the module.

4.3.4 Diafiltration

To increase WP purity during WP concentration by UF, diafiltration (DF) is employed, in which water is continually added to the retentate while lactose and minerals are simultaneously removed in the filtrate (De Wit *et al*., 1983; Zydney, 1988; Daufin *et al*., 2001). This is commonly done in constant-volume mode where water or buffer is added to the retentate at the same rate as permeation. There is an optimum protein concentration in the retentate at which to perform DF where the trade-off between permeate flux and the number of diavolumes is balanced and only the minimum membrane area or process time is necessary (Millipore, 2003; Glover, 1985). Using 20 kDa-MWCO PSf membrane sheets, Nilsson (1988) found that, in the UF of reconstituted WPC-80, the relative flux reduction (RFR) increased with protein concentration and then plateaued at about 3.2% protein concentration in the retentate. Beyond this concentration, the RFR increased sharply. Cheryan and Kuo (1984) showed that at 335 kPa TMP and 50 ºC, the flux approached a minimum when the

retentate reached about 3% protein concentration using PSf membrane in SW configuration while the flux in the HF was four times higher. Therefore, it appears reasonable to carry out DF in the SW when the protein concentration in the retentate is about 3% before going to second-stage UF using HF module.

4.4. MATERIALS AND METHODS

4.4.1. Pilot-scale production of LVWPI: Recovery and concentration of virgin whey

Step 1: Recovery of virgin whey by microfiltration.

The MF system used was a Tetra Alcross™ megaloop-38 (Tetra Pak Inc., Vernon Hills, IL, USA), which consisted of 38 Membralox® ceramic membrane elements (Pall Corporation, Deland, FL, USA) with nominal pore diameter of 0.1 μm. The elements were 1020 mm long giving an effective filtration area of 9.2 m^2. The process, which involved concentration of slightly acidified skim milk 8 times under uniform transmembrane pressure (UTMP) at 50 °C, was detailed by Ardisson and Rizvi (2004). The MF permeate, which was the VW, was collected and held in a jacketed stainless steel vat, gently stirred at 45 °C before using as feed stream to the UF system immediately after the MF process. Different amounts of skim milk were used to collect different amounts of VW (950 kg, 1025 kg, 1125 and 1325 kg) in order to vary the F to A ratio for the optimization procedure.

Step 2: First-stage ultrafiltration and diafiltration using spiral wound membrane.

The first stage of VW concentration was carried out using S4-HFK-131-VSV PSf in SW configuration from Koch Membrane Systems, Inc. (Wilmington, MA, USA) with MWCO of 10,000 and an effective filtration area of 5.9 m^2. The UF feed stream, which was the VW, had a pH of 6.1 with total solids (TS) of 5.31% (w/w),

about 6% of which was WP. The average cross-flow velocity in the SW module was 0.50 m s^{-1}. The average pressure drop along the length of the module, TMP and temperature were maintained at 275 kPa, 338 kPa and 45 °C, respectively. Filtration was continued until a MCF of about 13 was reached. DF then followed using four diavolumes of phosphate buffer to maintain the pH at 6.1. The number of diavolumes, which was based on the amount of the 13x concentrated SWM retentate, was pre-calculated to find the minimum DF time, minimum diavolumes and maximum flux that will give at least 90% (w/w) WP purity (dry basis) in the final retentate. Results of previous test runs were used for these calculations.

Step 3: Second-stage ultrafiltration using hollow fiber membrane.

The second stage of UF concentration was done using CTG, 3" HF-25-43-PM10 in HF configuration from Koch Membrane Systems, Inc. (Wilmington, MA, USA) with a MWCO of 10,000 and a total effective filtration area of 2.9 m^2. Immediately after DF, the SW module retentate was fed to the HFM module operating at 45 °C and an average crossflow velocity of 2.02 m s^{-1}. The pressure drop along the length of the module and TMP were maintained at 130 kPa and 235 kPa, respectively, until the MCF was about 5, giving a total MCF of about 65, to give the target of at least 90% WP (w/w, dry basis) in the final LVWPI. The weight of the permeate streams from the SW and HF modules was recorded at 10-minute intervals to determine permeate mass flux. The pH and temperature of the retentate were also recorded at the same time interval. The schematic of the LVWPI production is shown in Figure 4.1 and the operating parameters are summarized in Table 4.1.

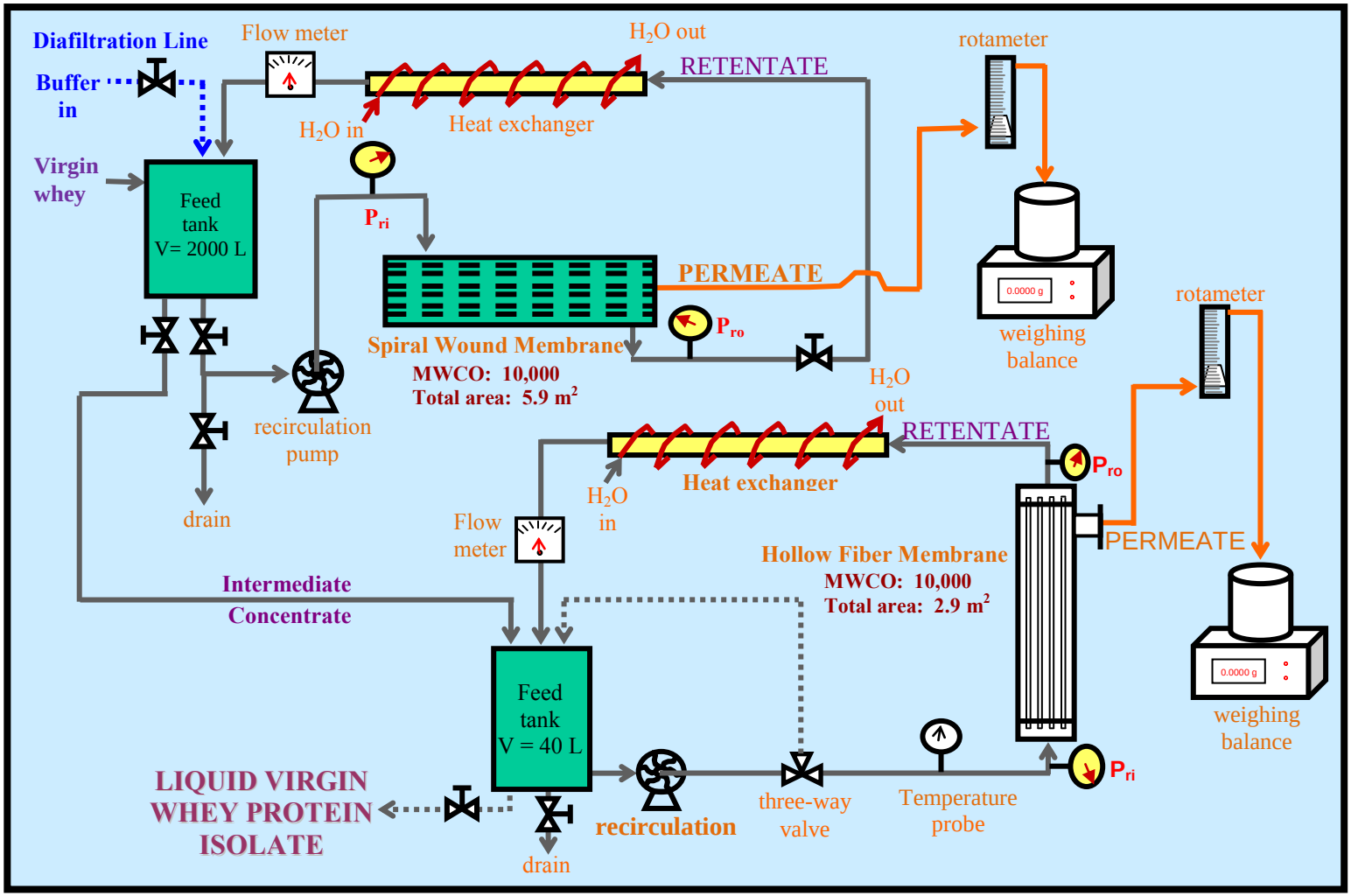

Figure 4.1. Schematic diagram of the ultrafiltration system used in the production of liquid whey protein isolate (LVWPI). The system consisted of polysulfone membranes in spiral wound and hollow fiber configurations in series.

Table 4.1. Operating conditions in the spiral wound and hollow fiber modules in the ultrafiltration of virgin whey to produce liquid virgin whey protein isolate.

Parameters	MF Tetra-Alcross	UF spiral wound module	UF hollow fiber module
Feed pH	6.0	6.1	6.1
Average temperature, °C	50.2	45.3	45.8
P_{inlet}[a], kPa	372	475	300
P_{outlet}[a], kPa	283	200	170
Average TMP, kPa	101[b]	338	235
Average crossflow velocity, m s^{-1}	0.5	0.5	2.0
% total solids in feed	9.19[c]	5.31[d]	8.26[e]
Final mass concentration factor	~8	~13	~5[f]
Clean water flux, kg h^{-1} m^{-2}	200	62.2	94.1
Membrane area, m^2	9.2	5.9	2.9

[a] Pressures in the retentate side.
[b] Uniform transmembrane pressure.
[c] GDL-acidified (pH 6) skim milk.
[d] Virgin whey.
[e] The feed is the 13x concentrated and diafiltered retentate from the SWM.
[f] Using the 13x concentrated and diafiltered retentate from the SWM.

4.4.2. Compositional analyses

The composition of VW and the UF retentate and permeate streams was determined following the AOAC (2000) protocol unless otherwise specified. The % TS was determined by drying in an oven at 100 °C for four hours (AOAC, 2000; 33.2.44, 990.20). Total nitrogen was determined by Kjeldahl method (AOAC, 2000; 33.2.11, 991.20) and the true protein was obtained after correction for non-protein nitrogen (NPN) (AOAC, 2000; 33.2.12, 991.21) using a protein conversion factor of 6.38. The sample size was adjusted so as to contain similar absolute amount of

protein as milk as recommended in the procedure, taking into account the concentration factors at different stages in the process. The true protein fraction was taken as equal to whey protein fraction. All determinations were done in quadruplicates.

4.4.3 Statistical analyses

All statistical analyses were done using MINITAB® release 14 statistical software (State College, PA, USA).

4.5. RESULTS AND DISCUSSION

4.5.1 Process analysis of the pilot-scale production of LVWPI

The SW flux vs. time data show two distinct segments: the region of rapid flux decline and the pseudo-steady state region where a flux plateau was apparent (Figure 4.2). The TMP of 338 kPa was only slightly higher than the critical TMP of

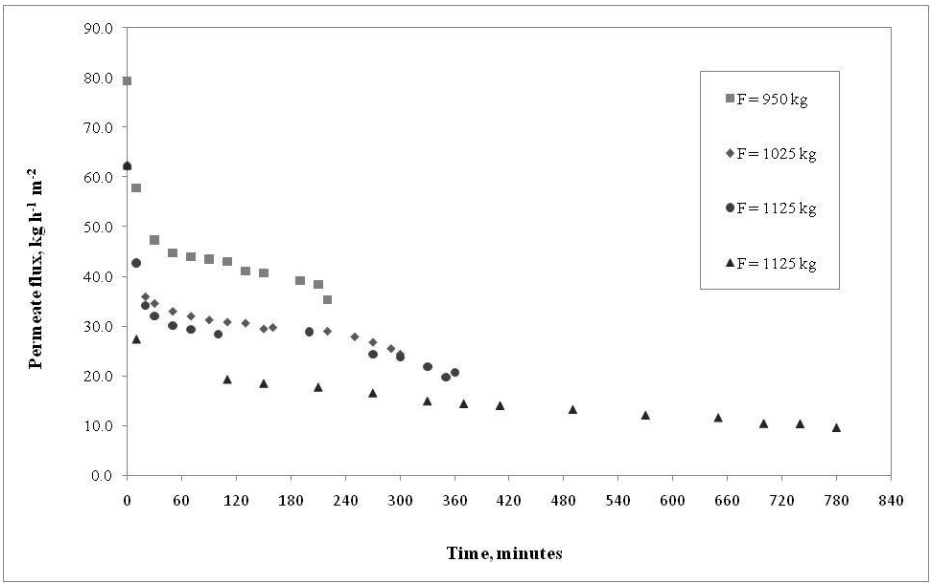

Figure 4.2. Flux history in the ultrafiltration of different quantities of virgin whey using 5.9 m^2 of polysulfone membrane in spiral wound configuration at pH 6.1, 45 °C and 338 kPa transmembrane pressure.

335 kPa found by Cheryan and Kuo (1984) in concentrating cottage cheese whey using PSf membrane, also in SW configuration. For the same feed quantity of 1025 kg VW, lower TMP of 225 kPa was also investigated, and resulted to lower permeate flux (Figure 4.3). Thus, TMP of 338 kPa was utilized in the study.

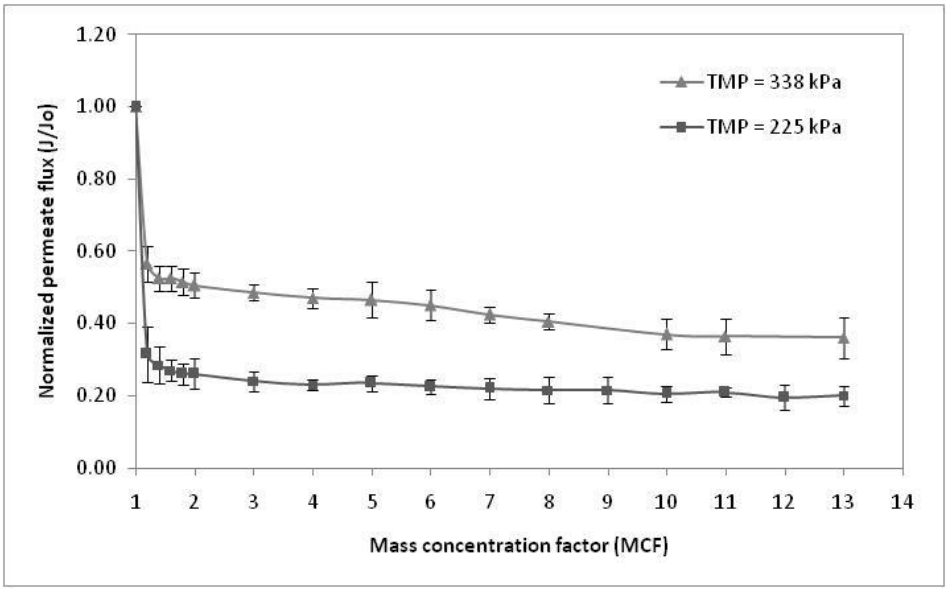

Figure 4.3. Variation of permeate flux with mass concentration factor using polysulfone spiral wound membranes at 45 °C and transmembrane pressures of 338 kPa and 225 kPa using 1025 kg of virgin whey as feed. Error bars are based on standard deviation of duplicates.

For 1025 kg VW feed, starting from a clean water flux of 62.2 kg hr^{-1} m^{-2}, the average permeate mass flux in the SW module was 42.9 kg hr^{-1} m^{-2} during the first 10 minutes of operation. For the same effective filtration area of 5.9 m^2, the average mass flux varied with the feed quantity, F (Table 4.2). Over the next 30 minutes, the permeate flux declined with time and plateaued to its pseudo steady-state value, which decreased with increase in F/A ratio, until a MCF of about 8 was reached. The flux then declined further as the MCF reached 13 and the retentate TS reached about 3% (w/w). The viscosity of the retentate, measured at 20 °C, increased from 1.57 to 2.13

mPa-s. This decreased the Reynolds number (N_{Re}) from 364 to 271 (Table 4.3), maintaining laminar flow in the retentate side.

Table 4.2. Variation of flux with feed quantity in the ultrafiltration of virgin whey in spiral wound polysulfone membrane at pH 6.1, 45 °C and TMP of 338 kPa to reach 13x mass concentration factor.

Feed, kg	F/A, kg feed per m^2 membrane area	Average initial flux[a], kg m^{-2} h^{-1}	Average flux[b], kg m^{-2} h^{-1}	Flux plateau, kg m^{-2} h^{-1}	Processing time, min
950	161	51.8	41.7	40.6	240
1025	174	37.8	30.1	30.0	330
1125	191	36.3	25.9	25.2	395
1325	225	23.8	14.8	14.0	885

[a] Average permeate mass flux during the first 30 minutes of operation.
[b] Average permeate mass flux all throughout the operation.

The low crossflow velocity of 0.50 m s^{-1}, which corresponded to a moderate longitudinal ΔP of 275 kPa in the SW module, allowed the maintenance of shear stress, τ_w, at the membrane wall of about 178 Pa. The shear stress was calculated using the suitable equation for laminar flow:

$$\tau_w = \frac{d_h(P_i - P_o)}{4L} \qquad (4.1)$$

where d_h is the hydraulic diameter of the SW module flow channel, L is the length of the membrane, and P_i and P_o are the inlet and outlet pressures in the retentate side of the SW module, respectively. The shear stress may have been sufficient in maintaining a balance between the rates of particle erosion and particle deposition on the membrane surface, so that no massive net deposition of solids was occurring on

Table 4.3. Lactose reduction and changes in the flow properties of the feed and retentate streams in the polysulfone spiral wound and hollow fiber membrane modules in the production of LVWPI at 45 °C and 338 kPa transmembrane pressure using 1025 kg of virgin whey as feed .

Parameter	Spiral wound			Hollow fiber
	Feed	Retentate		Retentate
		Before DF	After DF	
Density[a,c], g mL^{-1}	1.04	1.07	1.05	1.11
Viscosity[a,c], mPa-s	1.57	2.13	2.01	11.65
Reynolds Number (N_{Re})[b]	364	271	285	52
% total lactose reduction[c]	--	88.63	98.48	99.85

[a] Measured at 20 °C.

[b] $N_{Re} = \frac{D\bar{v}\rho}{\mu}$ where: D = hydraulic diameter of flow SW flow channel (0.001092 m); $\bar{v}$ = crossflow velocity (0.5 m s^{-1}); ρ = density, kg m^{-3}; μ = viscosity, Pa-s.

[c] Average of duplicates.

the membrane surface as evidenced by the quasi steady-state permeation, which continued even as the protein concentration in the retentate increased steadily (Figure 4.4). Maintaining quasi steady-state permeation even at moderate shear stress may have been the result of the absence of massive protein-protein interactions in VW, which is characteristic of native globular proteins (Tanford, 1961). In cheese whey UF, extensive protein-protein interactions commonly leads to aggregation and eventually results to rapid membrane fouling as the protein concentration in the retentate increased (Marshall *et al.*, 1993).

At the end of the flux plateau in the SW module as MCF of 13 was reached, DF commenced. During this period, the flux increased, remained almost constant at an average value that decreased with increase in F/A, and eventually decreased towards the end (Figure 4.5). After DF, where removal of lactose in the SW module

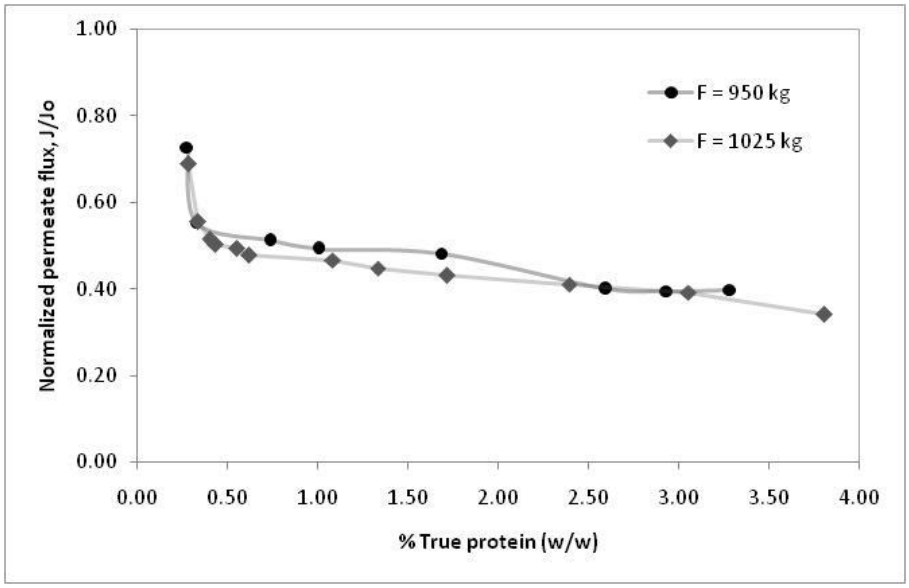

Figure 4.4. Variation of flux with true protein concentration in the ultrafiltration of virgin whey using 5.9 m^2 of polysulfone membrane in spiral wound configuration at pH 6.1, 45 °C and 338 kPa transmembrane pressure.

retentate was about 98% regardless of F/A, the %TS decreased by as much as 26% while the % (w/w) true protein increased by as much as 66%. On the other hand, the retentate viscosity, measured at 20 °C, remained approximately constant at about 2.12 mPa-s even as considerable changes in its composition occurred (Table 4.3). Such observation is in agreement with the findings of Morison and Mackay (2001) and Mleko *et al*. (2003) that although the proteins had the most influence in a WP solution viscosity, the contribution of lactose and mineral fractions is considerable. Before DF, the contribution of lactose and minerals to viscosity was substantial, and after their removal during DF, the viscosity remained constant due to the increased concentration of proteins. However, the viscosity remained low at the end of the DF process even as the % true protein (w/w) increased to about 6.33 (80%, dry basis), indicating that protein-protein interaction in the VW continued to be insignificant to manifest a

considerable increase in the retentate viscosity. This also indicated that the filtration process may not have affected the native conformation of the WP.

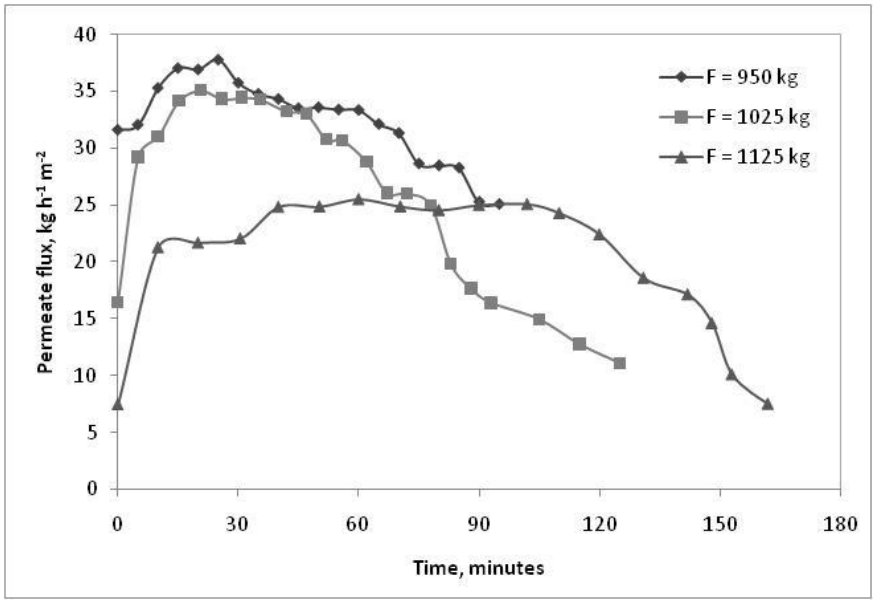

Figure 4.5. Flux history during diafiltration of pre-concentrated virgin whey in the spiral wound membrane at pH 6.1, 45 °C and 338 kPa transmembrane pressure for about 95% removal of lactose.

The second UF stage of LVWPI production using HF module showed exponential flux decay with time all throughout the process, regardless of F/A (Figure 4.6). The %TS increased more than three times, in which about 90% was true protein. The viscosity, on the other hand, went up by about six times to 11.65 mPa-s, measured at 20 °C. The crossflow velocity of 2.02 m s^{-1}, the ΔP along the length of the HF module of only 130 kPa, which corresponded to a wall shear stress of about 56 Pa, may have contributed to the lower mass flux in HF module. The wall shear stress, which was calculated by equation (1) using HFM dimensions, must have been too low to dislodge foulants on the membrane surface (Grandison *et al*., 2000), especially as the viscosity of the retentate also rapidly increased as the WP were concentrated.

These settings, however, were maintained to protect the proteins from extensive shear, which a number of workers have found to cause WP denaturation during the UF of cheese whey as the retentate was concentrated to high MCF (Morr & Ha, 1993).

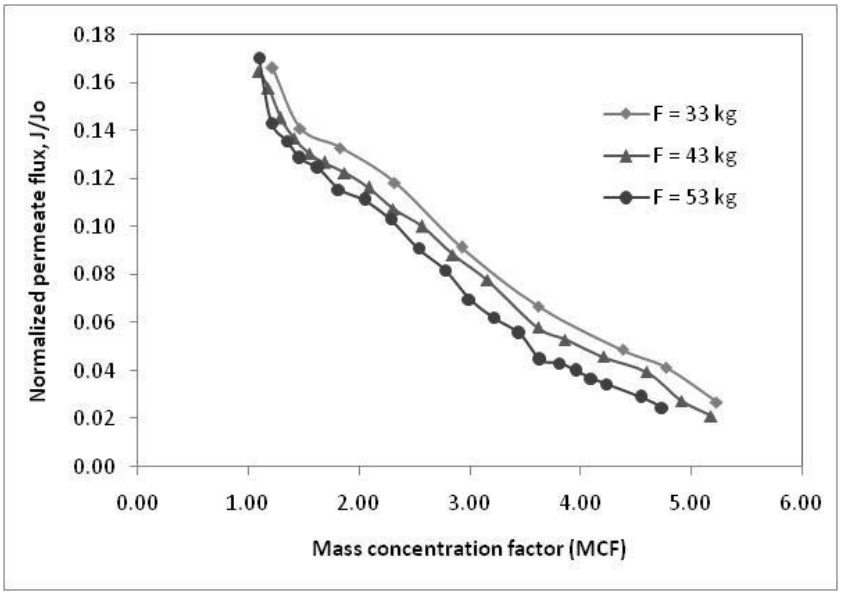

Figure 4.6. Variation of flux with mass concentration factor in the ultrafiltration of pre-concentrated and diafiltered virgin whey using 2.9 m^2 polysulfone membrane in hollow fiber configuration at pH 6.1, 45 °C and 130 kPa transmembrane pressure.

Aside from LVWPI produced at the end of the second-stage UF, the UF/DF process produced two types of liquid whey protein concentrates (LVWPC): (1) LVWPC-34, produced at the end of the first-stage UF, and (2) LVWPC-80, produced at the end of the SW module retentate DF. The changes in the composition of virgin whey as it was concentrated to produce LVWPI are shown in Table 4.4.

4.5.2 Energy consumption

Although membrane technology is less energy intensive than most separation

Table 4.4. Changes in the composition of virgin whey (VW) when concentrated in the ultrafiltration with diafiltration (UF/DF) system to produce liquid virgin whey protein isolate (LVWPI) at pH 6.1 and 45 °C.

Component	**% Composition (w/w)**			
	VW	Before DF[d]	After DF[d]	LVWPI[e]
Total solids[a]	5.31 ± 0.04	11.21 ± 0.04	8.26 ± 0.03	26.13 ± 0.16
True Protein[a,c]	6.03 ± 0.05	33.95 ± 0.01	76.67 ± 0.02	90.78 ± 0.70
NPN[a,c]	3.28 ± 0.01	2.20 ± 0.09	3.53 ± 0.14	4.92 ± 0.13
Fat[a,c]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lactose[b,c]	83.72 ± 0.18	58.59 ± 0.31	10.60 ± 0.42	1.60 ± 0.63
Ash[a,c]	6.97 ± 0.07	5.26 ± 0.12	9.20 ± 0.14	2.72 ± 0.06
Water[a]	94.69 ± 0.04	88.79 ± 0.04	91.74 ± 0.03	73.87 ± 0.16

[a] Mean of quadruplicates ± standard deviation.
[b] Calculated by difference.
[c] Dry basis.
[d] Using spiral wound membrane at 338 kPa transmembrane pressure.
[e] Using hollow fiber membrane at 130 kPa transmembrane pressure.

and concentration processes, energy consumption is still a major consideration for process feasibility. The major energy consumption in the UF of VW is that for pumping the feed into the membrane module and circulating the retentate from the feed tank within the feed tank-membrane loop (Lo *et al*., 1997). Assuming that changes in potential and kinetic energies are negligible, mechanical energy balance around the module yields:

$$W_s = \upsilon(-\Delta P) + \Sigma F \tag{4.2}$$

where W_s is the shaft work by the pump, υ is the specific volume of the feed or retentate, which is equal to the reciprocal of density, ΔP is the pressure drop along the length of the module and ΣF are the friction losses by VW as it flows in the module. ΣF was calculated using the Fanning-Darcy equation:

$$\Sigma F = \frac{f' \bar{v}^2 \Sigma\left(L/D\right)}{2g_c} \tag{4.3}$$

where L is the length of the module, D is the diameter of the flow duct in the spiral wound module, which was assumed to be a rectangular duct, $\bar{v}$ is the average crossflow velocity, g_c is the flow constant, which is equal to 1.0 when SI units are used, and f' is the friction factor, which for laminar flow conditions is calculated as:

$$f' = \frac{64}{N_{Re}} \tag{4.4}$$

Using the conditions used in the first-stage UF where SW module was used, W_s, expressed as J per kg of feed was calculated for different F/A values. Results of calculations estimated W_s as 264 J kg^{-1}. The actual W_s, which was calculated

assuming 70% efficiency of the pump was 377 J kg^{-1}. The energy requirement for pumping, expressed as J per kg permeate, was calculated considering permeate flux values at different F/A. The average permeate flux was calculated using flux values

flux values predicted by the flux model derived previously for UF of VW:

$$J = m\frac{F}{A}\left(\frac{1-\frac{1}{MCF}}{t}\right) \tag{4.5}$$

where J is the permeate mass flux (kg h^{-1} m^{-2}), m is the long-term fouling parameter, which for VW is equal to 0.89, F/A is the initial fouling parameter (kg feed per m^2 membrane area), MCF is the mass concentration factor at any time, t (minutes). The MCF values were calculated using:

$$MCF = 1 - \frac{[J_o A(b+1)]^{\frac{1}{b+1}}}{F} t^{\frac{1}{b+1}} \tag{4.6}$$

where b = 0.12 for VW, J_o (kg permeate per hour) is the initial permeate flux, which can be estimated by:

$$J_o = p\left(\frac{F}{A}\right)^s \tag{4.7}$$

where s = -2.11 and p = 5.81 x 10^4 kg permeate per hour per kg feed. For a given F/A, J_o was calculated and used in equation (4.6) to estimate the corresponding MCF at different values of t. The MCF values were then used in equation (4.5) to predict J as a function of t. The average flux was then calculated for a given value of F/A.

The pumping energy requirement per kg of permeate, E_{pp}, was almost constant at 407 ± 4.7 J for F/A values between 161 and 225 (F = 950 to 1325), and was calculated using:

$$E_{pp} = \left(\frac{W_{s,actual}}{Jt}\right)\left(\frac{F}{A}\right) \tag{4.8}$$

where t is the time needed to reach MCF of 13. However, due to the variation of average permeate flux and processing time to reach MCF of 13 with F/A, the total pumping energy requirement, E_{total}, also varied with F/A. E_{total} was calculated using:

$$E_{total} = E_{pp}\left[\frac{F(1-MCF)}{MCF}\right] \tag{4.9}$$

where MCF = 13. The summary of calculation results are shown in Table 4.5. Higher

Table 4.5. Total pumping energy requirement in concentrating different amounts of virgin whey (VW) to mass concentration factor of 13 using 5.9 m^2 polysulfone spiral wound membrane (molecular weight cut-off of 10 kDa) at 45 °C and 338 kPa transmembrane pressure.

VW Feed, kg	F/A, kg feed per m^2 membrane area[a]	Average permeate flux[b], kg h^{-1} m^{-2}	Pumping energy requirement, J
825	140	51.13	3.15 x 10^5
950	161	37.60	3.54 x 10^5
1025	174	31.75	3.91x 10^5
1125	191	25.81	4.24 x 10^5
1325	225	21.55	4.93 x 10^5

[a] For a membrane area of 5.9 m^2.
[b] Predicted by equations (5), (6) and (7).

VW throughput, or higher F/A values, required higher pumping energy to reach MCF of 13. It can, therefore, be expected that processing cost will be higher at this condition. However, processing cost also involves equipment cost and, in this case, the membranes constitute the major equipment cost.

Similar analyses were carried out for DF and the second-stage UF using HFM to evaluate the sum of pumping energies, ΣE, for the entire process. Because not all the retentate can be recovered from the SWM, only a fraction of the diafiltered SWM retentate was fed into the HFM. The results, which are summarized in Tables 4.6 and 4.7 indicate that the total energy requirement for pumping increases with F/A increase.

Table 4.6. Total pumping energy requirement in the diafiltration of different amounts of pre-concentrated virgin whey as feed using 5.9 m^2 polysulfone spiral wound membrane at 45 °C and 338 kPa transmembrane pressure.

Feed, kg	13x SWM retentate, kg	F/A, kg feed per m^2 membrane area[a]	Average permeate flux[b], kg h^{-1} m^{-2}	Pumping energy requirement, J
825	63.46	53.78	38.28	1.16×10^5
950	73.08	61.93	32.47	1.33×10^5
1025	78.85	66.82	26.60	1.44×10^5
1125	86.54	73.34	20.86	1.71×10^5
1325	101.92	86.38	16.12	2.69×10^5

[a] For a membrane area of 5.9 m^2.
[b] Predicted by equations (4.5), (4.6) and (4.7).

4.5.3 Process economics

In this study, the cost of processing was assumed to be the sum of pumping energy cost and membrane replacement cost, which are considered the major

processing cost. In the previous section, it was shown that higher F/A involves higher pumping energy requirement. So, if F/A is reduced to lower pump energy consumption, higher membrane cost would be incurred. Therefore, a truly optimized UF processing system is one where the two major costs are balanced.

Table 4.7. Total pumping energy requirement in producing LVWPI from different amounts of pre-concentrated virgin whey using 2.9 m^2 polysulfone hollow fiber membrane at 45 °C and 130 kPa transmembrane pressure.

SWM feed, kg	HFM feed, kg	F/A, kg feed per m^2 membrane area[a]	Average permeate flux, kg h^{-1} m^{-2}	Pumping energy requirement, J	ΣF, J
950	33	11.38	19.63	4.39×10^3	4.92×10^5
1025	42	14.48	12.97	5.70×10^3	5.30×10^5
1125	53	18.28	7.73	9.60×10^3	6.04×10^5
1325	63	21.72	4.23	1.81×10^4	7.79×10^5

[a] For a membrane area of 2.9 m^2.

To determine the total cost of processing, energy cost was assumed to be \$0.05 per kW-h while membrane cost was taken as \$200 per m^2 membrane area per year (Lo *et al*., 1997). Using different values of F/A to attain the desired concentration of WP in the retentate, the corresponding total costs of processing were determined. The optimal F/A was taken as that value where the total cost was minimum. Figure 4.7 shows that to reach MCF of 13 in the SW module, F/A of 150 gives the minimum total cost of \$1.69 per kg of feed. The F/A used in this work ranged between 161 and 225. Therefore, for a filtration area of 5.9 m^2, smaller feed quantity may reduce the total

processing cost.

Since the DF is based on the final retentate from the SW module, and that the number of diavolumes used was calculated based on the desired purity of the final product, no optimization procedure was followed for the cost of DF processing. Instead, the cost of DF was calculated based on the optimum F/A value of 150 in the first-stage UF using SW module. Calculation gives $3.78 per kg of feed. For DF, feed quantity is the sum of the SW module retentate and the four diavolumes of buffer used. The cost is high for this step because of low throughput, with low F/A value of 58. Therefore, even if the cost of pumping is low at $0.42 per kg of feed, the membrane cost is $3.36 per kg of feed.

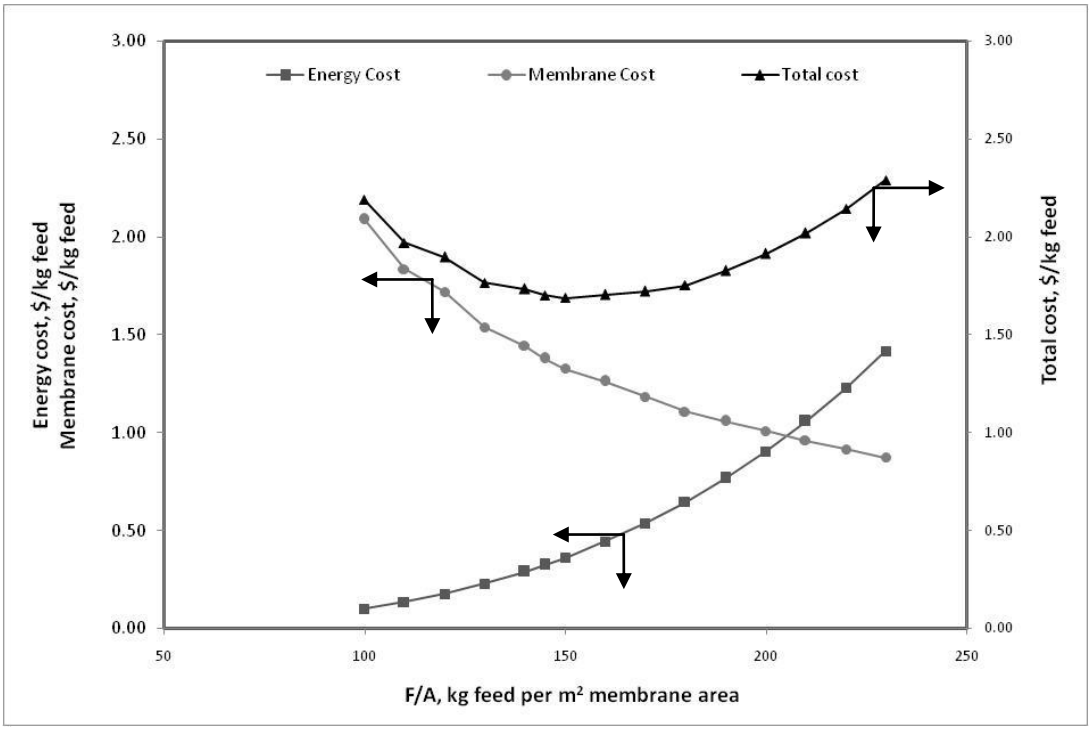

Figure 4.7. Optimization curves for the concentration of virgin whey to 13x using 5.9 m^2 polysulfone membrane in spiral wound configuration at pH 6.1, 45 °C and 338 kPa transmembrane pressure.

In the second-stage UF using HF module, equation (4.5) does not apply in predicting permeate flux. In the optimization procedure, the following equation (Merin and Cheryan, 1980) was used to estimate the average permeate flux:

$$J = J_o V^{-b} \tag{4.10}$$

where V is the cumulative amount of permeate collected at any time, t, J_o is a fouling parameter calculated using equation (4.7), with the experimentally determined constants, p and s, equal to 0.526 kg permeate per hour per kg feed and 0.12, respectively, and b is the long-term fouling parameter, which can be estimated using:

$$b = p'\left(\frac{F}{A}\right)^{s'} \tag{4.11}$$

where s' and p' are experimentally determined constants equal to 0.301 and 0.207, respectively, for 13x concentrated and diafiltered VW.

Permeate fluxes were predicted at different values of F/A, and were used together with the mechanical energy balance equation, and the energy and membrane cost to calculate the total processing cost. Using 1000 kg of feed as basis, the optimal F/A was found to be 420. In this work, the F/A value in the HF module ranged between 11.4 and 18.3. Greater F/A was not tried because the amount of retentate from the SWM was small compared to the large filtration area of 2.9 m^2 of the HF module used. Nevertheless, results of the experiments allowed the determination of the optimal F/A.

4.6. CONCLUSION

The process economics of LVWPI production depends on a balance between the total energy requirement and membrane cost. Findings in this study showed that

F/A affected permeate flux, which in turn, affects pumping energy requirement and membrane cost. Therefore, F/A is a useful design parameter, and an optimal UF system for LVWPI production can be obtained from an optimal F/A value. For the two-stage UF with DF process proposed for the production of LVWPI in this study, the optimal F/A in the first-stage UF is 150, while in the second-stage UF, optimal F/A is 240.

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CHAPTER 5

Production of liquid virgin whey protein isolate in the Philippines: a proposed technology transfer model

5.1. ABSTRACT

A technology transfer model for the production of liquid virgin whey protein isolate (LVWPI) is proposed. The source of technology is the United States of America through the food process engineering research group of the Cornell University Food Science Department, and the recipient is the Philippines. The technology transfer will benefit the Philippine dairy industry and Filipinos suffering from protein energy malnutrition (PEM). The model involves a tri-institution technology transfer channel, composed of the Academia, represented by the University of Santo Tomas (UST), the government, represented by the Department of Agriculture National Dairy Authority (DA-NDA), and the industry, represented by San Miguel Corporation (SMC). UST will be responsible in directly assimilating the technology and disseminating information to NDA and SMC. NDA will be responsible in bringing the technology to the grassroots, consisting of dairy cooperatives and entrepreneurs, and SMC will be responsible in providing suitable food vehicles for the highly nutritious native whey proteins in LVWPI to the PEM-affected Filipinos. Both NDA and SMC will provide research funding for research efforts of UST. The model is envisioned to result in improved and sustained productivity of the Philippine dairy sector, dynamic research in dairy technology at UST, and improved health and well-being for Filipinos suffering from PEM.

5.2. BACKGROUND

The Philippines produces only about 1% of its milk and milk products requirement, the rest is imported from other countries such as New Zealand, Australia and the United States of America (IDF Bulletin, 1997). For safety and long shelf-life, the imported milk is either ultra-high temperature (UHT) sterilized liquid milk or spray dried powdered milk. Due to the cooked taste and off-flavor associated with these types of milk products (Bandler & Barnard, 1984), Filipino children do not develop a strong liking for milk. These, the prevalence of lactose intolerance among Filipino adults, and the high cost of imported milk products are the primary reasons why a large number of Filipino population cannot benefit from the nutritional properties of milk. In 2001, the Food and Nutrition Research Institute (FNRI) reported that Filipinos consume only 44 grams per capita per day of milk, which constitutes only 5.5% of the total weight of a typical Filipino diet.

To meet the demands despite insuficient local milk productivity, the government set low importation tariff of 1 to 3% for milk and milk products (NDA, 2003). Therefore, for local milk and dairy-based products manufacturers, it is practical to import milk rather than rely on local produce. This and the lack of advanced milk processing technology in the country do not encourage the dairy sector to improve productivity and become more competitive. To address this, the Philippine Department of Agriculture (DA) through its implementing agency, the National Dairy Authority (NDA), launched aggressive efforts to improve dairy production by promoting dairying not just as a big-investment industry but also as an entrepreneurial activity. Presently, NDA funds are being spent in importing dairying animals that are distributed to dairy farmers in the major milk-producing regions in the Philippines, such as Bicol, Batangas, Nueva Ecija and Bulacan, through the dairy cooperatives in the regions (NDA Bulletin, 2007). This led to improved productivity in cattle farms,

which is estimated to have increased by 4.64% between 2005 and the first nine months of 2006 (NDA Bulletin, 2006). For the first quarter of 2007, the DA reported that the dairy sector posted an increase of about 3.87% increase in earnings for a total value of PhP 88.32 million from PhP 85.02 million in 2006 (Agricultural Statistics, 2007). This NDA-assisted improvement of productivity benefited school children from low-income families living in the dairying areas where milk feeding programs for children are conducted by the NDA with support from respective local governments. The milk used for these programs are obtained by NDA from dairy cooperatives-managed milk processing plants to provide the cooperatives with steady income.

Adding value to the local dairy produce for sustained productivity and manufacturing dairy products that are suitable for nutritional needs of Filipinos are now the bigger challenges. One way to meet these challenges is through active research in dairy processing and product development, which can be conducted in the Academia. Through government- and industry-assisted academic programs in this area, dairy science and technology research will pave the way to modern dairy processing and product development in the Philippines. At the same time, such programs can be the means to train local dairy scientists and technologists whose skills are critical in sustaining productivity. Therefore, sustainability can be achieved through coordinated efforts among the dairy sector, the government and the Academia.

5.3. RATIONALE

5.3.1 Protein energy malnutrition status in the Philippines

Together with micronutrient deficiency, protein-energy malnutrition (PEM) is one of the most prevalent forms of malnutrition in the Philippines (National Nutrition Council, 1999). Children, as well as pregnant and lactating women, are the most affected groups. In the 2003 nutritional status survey conducted by the Philippine

Department of Science and Technology's (DOST) FNRI, it was found that among 0-5 years old children, about 27.6% are underweight-for-age, 30.4% are stunted or short for their age, 5.5% are thin and 14% are overweight. The FNRI-DOST further found that these numbers are almost the same for the 6-10 years age group while 15.5% and 3.5% of pre-adolescent and adolescent age group are underweight and overweight, respectively. Among pregnant and lactating women, 26.6% and 11.7%, respectively, are underweight. The FNRI-DOST study suggested that such problem is a result of compounded nutrition problem where PEM is a major cause. This is because the protein consumption of the average Filipino is derived from cereals, mainly rice, containing proteins of low biological value (NNC, 1999). This has significant implications among growing children since protein of high biological value, such as those derived from animal sources, is needed to support growth and overall health (Black, 2003; Murphy & Allen, 2003). This is the foremost reason for the milk feeding program conducted by the NDA.

5.3.2 Nutritional benefits of whey proteins

Whey proteins, the serum proteins of milk, which are generally obtained and processed presently as co-product of cheesemaking, are high-quality proteins (Etzel, 2004). By virtue of their essential amino acids content, the biological value of whey proteins is high compared with that of other dietary proteins (Walzem *et al*., 2002). Among all protein sources, whey proteins contain the highest concentration of the branched-chain amino acids, L-isoleucine, L-leucine, and L-valine (Walzem *et al*., 2002), which are found to support numerous metabolic processes ranging from the fundamental role as substrate for protein synthesis to metabolic role as energy substrates, precursor for synthesis of alanine and glutamine and as modulator of muscle protein synthesis (Layman, 2003). The high sulfur-containing amino acid

content of whey proteins appears important to their ability to enhance immune functions and antioxidant status via modulation of the sulfur-containing tripeptide glutathione (Bounous & Gold, 1991). Tryptophan, another abundant amino acid in whey, is a precursor for the neurotransmitter serotonin. The relative surplus of some essential amino acids (lysine, threonine, methionine, isoleucine) in whey proteins, make them effective supplements to vegetable proteins, which often are limiting in those amino acids (Walzem *et al*., 2002). Virtually, every amino acid present in sweet-type whey, obtained from rennet type hard cheese like cheddar or Swiss cheese, exceeds Food and Agriculture Organization/World Health Organization (FAO/WHO) nutritional intake recommendations, both for children aged 2 to 5 and for adults (Walzem *et al*., 2002). Inclusion of these proteins, therefore, in the Filipino diet, through a suitable vehicle, may alleviate the problem on PEM.

Whey proteins, obtained at the end of the cheesemaking process, are presently utilized by the food industry in the powder form as whey protein concentrate (WPC), with up to 80% whey protein content, and whey protein isolate (WPI) containing, at least, 90% whey proteins. They are valuable as food ingredients, not only for their ability to aggregate and provide structure to foods, but because they are highly soluble over a wide pH range. These properties make them suitable for use in such applications as baked products and processed meats as well as sports beverages and liquid meal replacements (Walzem *et al*., 2002). Etzel (2004) suggested that one alternative to low-protein high-sugar beverages is to develop a high-protein low-sugar beverage having a slightly higher pH than most soft drinks. WPC and/or WPI, both rich sources of high quality protein, could be a suitable ingredient for this purpose. However, Etzel (2004) showed that beverages made from commercial WPI form sediment layers and turbid solutions at pH 4.0 – 5.5, a result of damage to the whey proteins during manufacture. The same author showed that beverages prepared using

WPI, produced by ion-exchange chromatography to minimize protein damage, do not show such disadvantages. The cost effectiveness of such production method, however, may need further evaluation for suitable applications to allow development of affordable whey protein-rich food products in developing countries, such as the Philippines. In an optimized process design, ultrafiltration with diafiltration (UF/DF) proves to be more cost-effective compared to ion-exchange in producing WPI (Barba *et al*., 2001).

5.4. TECHNOLOGY SUMMARY

5.4.1 The liquid virgin whey protein isolate

The variability in the composition and functionality of WPC and WPI, even when manufactured under similar conditions, hinders the full exploitation of the benefits of whey proteins (Patel *et al.*, 1990). The compositional differences and variations between acid and sweet whey (Schmidt, *et al*., 1984) from which WPC and WPI are produced have been pointed out as among the many factors that contribute to this problem. De la Fuente, *et al.* (2002), found that the differences in protein composition and functionality between different whey types are more related to the processes that are used in cheese or casein manufacture than to changes during the WPC manufacturing process. This means that if whey proteins are recovered prior to cheese making process, such compositional and functional variability may be reduced. If the proteins were concentrated by UF alone to a high concentration factor and obtained as a liquid concentrate as opposed to the traditional ultrafiltration-evaporation-spray drying manufacture of commercial WPC powder, the proteins may be obtained in their native state. The liquid concentrate may then be used directly for food production. By this approach, aside from allowing a more cost-effective

commercial production and utilization, the biological activities of the proteins in the concentrate are not diminished.

In Cornell University (CU), Brandsma and Rizvi (1999; 2001) developed a process of cheese manufacture that involves microfiltration (MF) combined with in-process pH adjustment of skim milk to produce highly concentrated retentate vastly depleted of Ca and whey protein. This method was used in developing the vatless manufacture of cheese (Ardisson-Korat & Rizvi, 2004). Aside from enabling the production of good-quality cheese, through this process, the whey can be collected as "virgin whey", free of fat, spores and bacteria with the proteins in their native state (Brandsma & Rizvi, 2001; Ardisson-Korat & Rizvi, 2004). It renders the whey composition to be invariant with the type of cheese made. The absence of cheese making foulants and the native state of the proteins in the whey stream obtained from this process allow the concentration of whey to a high concentration factor using UF by alleviating flux decline (Marcelo & Rizvi, 2008). Moreover, lactose, which when present in large amount can be detrimental to the quality of the whey concentrate, due the Maillard reaction, is reduced to negligible level by DF.

The CU's Food Process Engineering research group has recently developed a process to produce liquid virgin whey protein isolate (LVWPI) (Marcelo & Rizvi, 2008). The process involves the use of polysulfone UF membranes with 10 kDa molecular weight cut-off (MWCO). The UF system consists of two stages. The first stage uses a spiral wound module with a total filtration area of 5.9 m^2 while the second stage consists of a hollow fiber module with effective filtration area of 2.9 m^2. In the first stage UF, virgin whey is concentrated 13 times at 45 °C and 338 kPa transmembrane pressure. DF follows using four diavolumes of phosphate buffer. The pre-concentrated and diafiltered virgin whey then undergoes second-stage UF and concentrated five times, giving a total concentration of 65. The process produces three

different products: (1) LVWPC-34, obtained at the end of the first stage UF, (2) LVWPC-80, obtained after DF, and (3) LVWPI at the end of the second stage UF.

The LVWPI, which contained more than 25% total solids, more than 90% of which are native whey proteins, exhibited viscosity lower than either WPI or WPC-80 solutions with the same protein concentration (Marcelo & Rizvi, 2008). It does not form indiscreet aggregates even up to 80 °C for 30 minutes of heating at high protein concentration of 8 to 10% (w/w). These attributes are very appropriate in utilizing it as an ingredient in various tropical fruit juices largely consumed in the Philippines and produced by large local beverage companies, such as San Miguel Corporation (SMC). Aside from its nutritional benefits, its excellent functional properties may also be exploited in improving overall quality of existing products in various segments of the Philippine food industry, such as bakery, meat industry, noodles manufacture, confectionery, and the manufacture of baby foods.

5.4.2 Technology adoption proposal

It is proposed that the University of Santo Tomas (UST) food research team adopt the LVWPI production developed by the CU food process engineering research group using research facilities in the Thomas Aquinas Research Complex (TARC) of UST and milk produced in the Philippines. This will be done through the proposed technology transfer model. At the completion of the technology transfer, the acquired technology will then be utilized in developing suitable and commercially viable processes to enable Filipinos in wide income spectrum to benefit from the nutritional qualities of LVWPI. For instance, LVWPI can be used commercially in low-cost non-alcoholic beverage formulations. These beverages, which are popular to school children and young adults in the Philippines, who are largely affected by PEM, can be a viable vehicle for the highly nutritious native whey proteins. The nutritional quality

of other low-cost popular food products in the Philippines, such as bakery products, and baby food and noodles manufactured in government-subsidized facilities that are mostly intended for the indigent groups of the Filipino population, can be augmented using locally produced LVWPI. Information on processes and products developed by the UST research team will be shared with the industry to realize commercialization. The same information will be disseminated to the NDA for the dairy sector's continuing education and possible adoption of the technology to existing cooperative-managed processing facilities to increase local milk value.

5.4.3 Key deliverables

The following are the key deliverables of the technology:

- Value addition to local dairy produce: The nutritious qualities and functional properties of the native whey proteins in LVWPI can be exploited in a number of locally produced food products, both by profit-oriented businesses and government-subsidized food manufacturing facilities, which will be available and affordable for the largely impoverished Filipino population. This will add value to locally produced milk.
- Incentive for higher productivity to the local dairy sector: The value addition to locally produced milk will provide incentive to dairy farmers and entrepreneurs to improve productivity. Also, since LVWPI has potentials for numerous applications in industries other than the food industry, such as the pharmaceutical industry, the demand for locally produced LVWPI in the Philippines may increase. This may provide incentive to dairy farmers to increase productivity, which may positively impact the economic status of the Philippine dairy industry.

- Improvement of Filipino health status: The commercialization of LVWPI production in the Philippines, either by profit-oriented businesses or dairy cooperatives or both, using locally produced milk may lead to cost-effective high-quality protein fortification of existing food products. In the long run, efforts may lead to fortification of Filipino staples, such as rice, which will reach a wider spectrum in the population. This will largely benefit the PEM-afflicted Filipino population.
- Venue for useful applied research in the Academia: Aside from coming up with output of practical value to the industry and the dairy sector, the process of assimilating the technology will provide practical training to UST research students. This will prepare them as skillful technologists invaluable to sustaining progress in the dairy industry.

5.5. THE TECHNOLOGY TRANSFER MODEL

In the proposed technology transfer model (Figure 5.1), the United States of America will be the source of technology and the Philippines is the recipient. The channel of transfer will be a tri-institution partnership among the Philippine government, through the DA-NDA, the dairy industry, represented by a major food products manufacturer in the country, SMC, and the Academia, represented by the UST Food Research team (Figure 5.2). As technology source, the USA is represented by the CU, through the Food Processing Laboratory of the Food Science Department. UST, as a channel, will be in direct contact with the CU through collaborative research and consultations. The UST food engineering research group, which will be composed of the chemical engineering and food technology research teams, will be responsible for two things: (1) assimilation of the technology and (2) spearheading

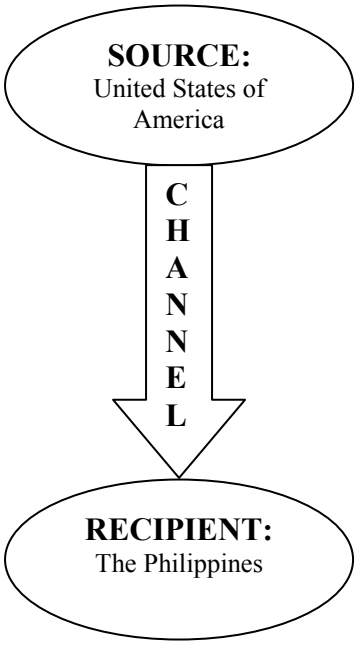

Figure 5.1. The technology transfer model.

research in developing viable schemes by which such technology could be utilized locally, in collaboration with the industry and the government agency in the tri-institution partnership.

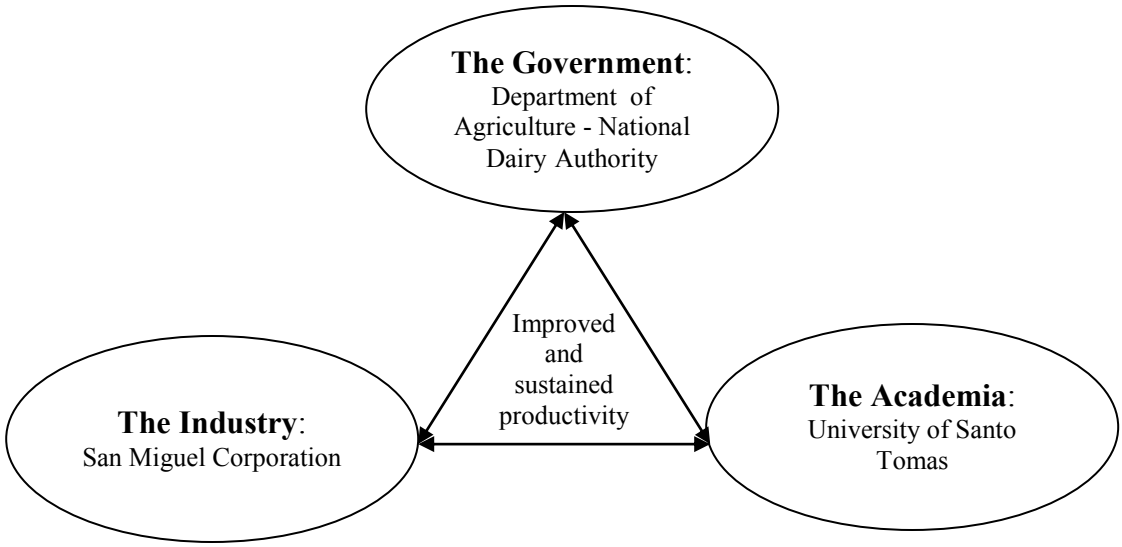

Figure 5.2. The technology transfer channel: tri-institution partnership.

The technology transfer will be carried out in a three-year, three-phase scheme as follows (Table 5.1):

Phase 1: Assimilating the technology and building research capabilities for dairy processing in UST under consultation with CU. This will involve recruitment of research personnel and student researchers in the graduate and undergraduate programs of UST, putting together the pilot-scale membrane facilities using university, industry- donated and DA-NDA-donated funds, training research personnel and students, and carrying out research works.

Phase 2: Information dissemination to the dairy sector by UST through the NDA. This will involve adopting the LVWPI production technology in cooperative-managed dairy processing zones around the country where milk is collected, pasteurized and made into traditional products, such as cheese, butter and dairy-based snacks.

Phase 3: Commercialization of whey protein-enriched products developed in TARC using locally produced LVWPI. Commercialization can be in small-scale through the dairy cooperatives or in large-scale through the industry.

Table 5.1. Time frame of technology transfer scheme.

Technology Transfer Phase	Year 1	Year 2	Year 3
Phase 1: Technology assimilation; membrane pilot plant commissioning			
Phase 2: Information dissemination; process and product development			
Phase 3: Product commercialization			

5.6. TECHNOLOGY SOURCE, THE TRI-INSTITUTION PARTNERS, AND THEIR ROLES

5.6.1 Cornell University

The Cornell University, through the food process research group of Prof. Syed Rizvi, will provide the technology for this project. The group developed the vatless manufacture of cheese through which virgin whey, the raw material for the LVWPI production, is obtained. The group also developed a process for the pilot-scale production of LVWPI. Along with the process, they also developed process optimization tools for straight-forward transfer of technology to the recipient. Through a research student who trained with the group and affiliated with UST, the technology transfer to the Philippines can be accomplished. Research output from such collaboration, such as publications or patents, will be shared by CU and UST groups in accordance to existing rules of the two institutions.

5.6.2 University of Santo Tomas

UST has recently built a food pilot plant in TARC, which will be dedicated to food process and product development, using locally available raw materials. Two research teams will be involved – the chemical engineering and the food technology teams. They will be responsible in planning and writing research proposals for funding arrangements with SMC and DA-NDA. Using the pilot plant space at TARC, the engineering research team will build its own MF-UF/DF pilot-scale facilities using university research funds and grants from SMC and DA-NDA. Once the technology is learned and the facilities are up and running, the processes in utilizing LVWPI as an ingredient in food products will be designed, starting from non-alcoholic fruit beverages, which are locally produced in the Philippines. The food technology research team will assist in product development, specifically, the formulation of the

products that will be of nutritional benefits to the target market. The same team will carry out sensory studies to make sure that the LVWPI-fortified products suit the Filipino palate.

The group will be responsible in sharing with and disseminating information to SMC and DA-NDA, and suggesting ways to adopt the technology in existing processing facilities. Training DA-NDA and cooperative-managed milk processing facilities personnel will be their minor responsibility, subject to availability of funding.

5.6.3 Department of Agriculture - National Dairy Authority

The creation of the NDA through the National Dairy Development Act of 1995, as the dairy industry policy and program implementing agency of DA, has increased milk production from 11 tons per day in 2001 to 21 tons per day in 2005 in NDA-assisted areas (NDA, 2005). This increase in productivity has benefitted some 94,000 indigent children who are participants in the milk feeding programs instituted as national nutrition program and sponsored by NDA (NDA, 2005). Aside from providing milk for the NDA's milk feeding program, other cooperative-managed milk processing facilities, such as the Northern Mindanao Federation of Dairy Cooperatives in Misamis Oriental, have ventured into producing commercial products such as milk bars in addition to traditional products, like cheese and butter. This significantly augmented the dairy farmers' income (NDA, 2005).

The NDA has been providing technical support to dairy farmers and entrepreneurs through continuing education programs, consultations and the use of NDA's testing laboratories for milk quality standardization. These aggressive efforts to augment milk production in the Philippines have opened up avenues in introducing value-added products, such as whey protein products, that will not only increase the

dairy sector's income, but also benefit the largely PEM-affected population.

In the tri-institution technology transfer channel, NDA will have two major roles:

(1) To provide assistance through research funding to UST for technology assimilation, and dairy process and product development-related research works. They will also aid in sourcing milk for the UST group's use.

(2) Being in direct contact with the dairy farmers and milk processing facilities operators, NDA will also serve as the conduit between the UST group and the dairy sector in completing the technology transfer from the source to the grassroots. Being the policy and program implementation arm of DA, this is deemed as an apt role for NDA.

5.6.4 San Miguel Corporation

The Philippine food and beverage industry is among the largest and the most diverse in Southeast Asia (Bernales, 2003). This is due primarily to the Philippine government's recent liberalization of the retail sector, among many other reasons. The continued growth of this sector allows wider diversification and entices Filipinos to spend more than half of their daily budget on food and beverages (Bernales, 2003). The leading local food companies, such as the food, beverage and packaging giant, SMC, and Republic Flour Mills (RFM) continue to aggressively compete in this liberalized market environment with new, healthier but affordable products (Lopez, 1996). RFM, for example, has been boosting its affordable "healthy beverages" (milk and juices) product line, targeting not only the A and B markets, but also the C and D markets (Visto, 2003). Even Wellex Group, which owns one of the biggest local packaging companies in the Philippines, recently put up its own food company, Philfoods, to primarily produce bottled drinking water, fruit juices, powdered juices

and cereal-based products like biscuits, instant noodles and various snacks at competitive prices (Visto, 2003).

SMC, the largest publicly listed company in the country and in the Southeast Asian region dominates the Philippine food and beverage market. It has been aggressively expanding locally and regionally. SMC recently acquired a 50% stake in the Australian juice company, Berri Ltd., which sells more fruits juices than any other company in Australia (Asia Pulse, 2004). SMC also owns and operates several facilities in China, Thailand, Vietnam, and other countries for its non-alcoholic beverage production (Rubrico, 2004). SMC's already huge beverage sales increased yet again by 15% in 2003 (Gallardo, 2003) while 33% of its total sales in 2004 were from its food products. It has most recently acquired National Foods, one of the largest food companies and the only national milk company in Australia (Calayag, 2005). In 2005, it was pushing to acquire 40% of fruit and juice maker Del Monte Pacific Ltd. from the Italian food group Cirio (Vila, 2005). In spite of the regionally expanding market of SMC, more than 86% of its total sales in 2004 were in its home market, the Philippines (SMC, 2004).

Founded in 1890, SMC is a long established, highly respected business giant in the Philippines and in the Asia-Pacific region (Pulse Asia, 2004), and has always been every Filipino's pride. Presently, it operates more than 100 facilities in and out of the country and employing more than 26,000. Its good reputation to the Filipino consumers does not only root from its excellent-quality and yet affordable products but also from its continued participation in nation building. It has assumed social responsibility through its own initiatives: promoting self-reliance to marginalized Filipino communities by providing business opportunities and lending financial assistance to Filipino entrepreneurs, all in an effort to alleviate poverty in the country. Through its San Miguel Foundation, Inc., SMC has taken an active role in social

development by, among many others, providing financial assistance to poor but deserving students especially those in the agricultural and vocational-technological field, promoting the advancement of science and technology research and the dissemination of the applications of these research findings in the country. It has also been providing support to the government's civic programs.

With all its sustained corporate growth and commitment to excellent product quality and social responsibility by improving the health and wellness of the Filipino, SMC may allow its non-alcoholic beverages, widely accepted by Filipinos, as vehicle in delivering the high-quality whey proteins to the Filipino's diet to help alleviate PEM. The wide variety of SMC's popular product lines in the dairy-based, meat and snacks categories, which can be used as vehicles for fortification, presents more opportunities in delivering the health benefits of whey proteins to most Filipinos. SMC's investment on product promotions and advertisements will help in promoting awareness about the health benefits of whey proteins in the country. Therefore, the major role of the industry, as represented by SMC, will be to bring the benefits of the technology transfer to the consumers.

5.7. BENEFICIAL FEATURES OF THE MODEL

The technology transfer model proposed, where a tri-institution channel is involved, may prove effective in sustaining the positive impact of the technology on the recipient (Figure 5.3). The inclusion of the Academia as a direct recipient of the technology, through an academic program, will not only ensure effective information dissemination to the industry but will also sustain research activities that may extend beyond the scope of the transferred technology. This will help the dairy industry thrive. The training of students in applied dairy technology research ensures constant

flux of skilled technologists who will make up reliable workforce in the local dairy industry in the future.

The involvement of the government as a technology transfer channel through the NDA, will ensure that the technology reaches the grassroots, which in this case are the dairy cooperatives and entrepreneurs that make up the agriculture dairy sector. Through joint efforts with UST, the continuing education of the dairy sector is ensured. The direct contact of NDA with the dairy sector and UST will ascertain effective dialogue that will help UST determine research activities relevant to the dairy sector. Being the policy and implementing arm of DA in dairy-related issues, results of these dialogues will help NDA determine effective policies and implementation strategies related to technology assimilation.

The inclusion of the industry as a technology transfer channel provides practical strategies in bringing the benefits of the assimilated technology to consumers from wide income spectrum, while maintaining positive economic impact. As a source of research funding for the Academia, the industry serves as the fuel to the technology transfer model. After technology assimilation, the industry serves as vehicle in bringing the product to the consumer and educating the public of the products' health benefits through infomercials.

5.8. MEASURES OF SUCCESS AND FUTURE OPPORTUNITIES

At the completion of technology transfer, success could be assessed using the following indicators:

- Dairy sector: The proportionate increase in productivity and income generation as a result of demands in LVWPI can be quantified.
- Nutritional benefits: LVWPI-fortified food products can be used in the dairy cooperatives-sponsored milk feeding programs for school children, which are in

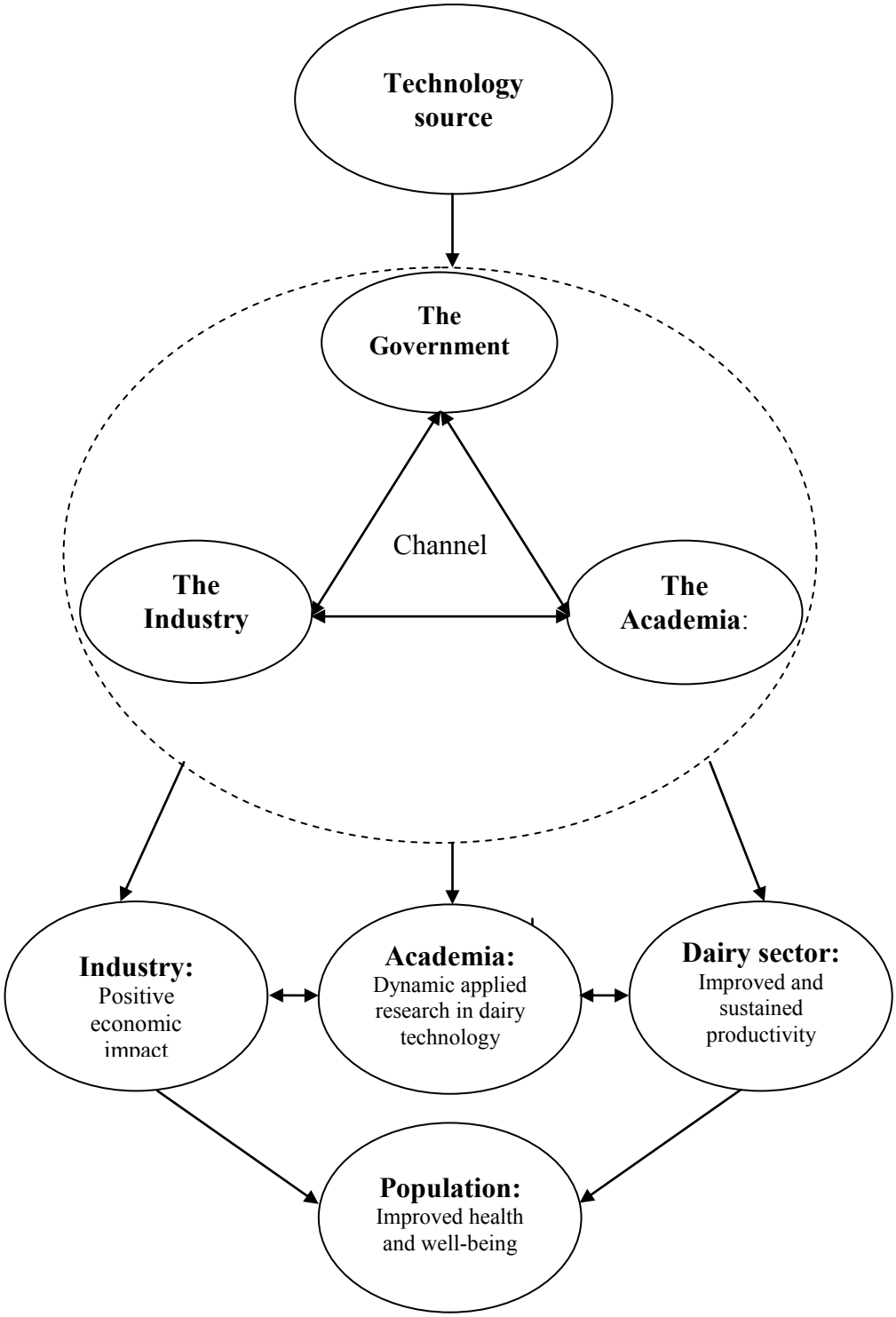

Figure 5.3. Beneficial features of the proposed technology transfer model.

collaboration with the Department of Health (DOH). The improvement in the nutritional status of the children as a result of LVWPI food fortification can be assessed using standard methods already established by DOH.

- Economic gains by the dairy industry: The increase in net sale of existing products fortified by LVWPI compared with the same products not fortified with LVWPI can be determined. Here, the expense for infomercials and other product promotion efforts must also be considered.
- Academia (the UST scenario): Success can be assessed by considering intellectual output and outreach activities with NDA. The growth of the dairy processing program at TARC after the two-year assimilation period, which includes collaborations with researchers in and out of TARC and by the increase in the number of student participants in the program, can also be used as additional indicator.

Another important feature of the model proposed is that at the end of the technology transfer scheme, the tri-institution channel can become a stand-alone partnership that may continue to explore more avenues in improving and sustaining the dairy industry in the Philippines. Collaborations can be expanded to the involvement of more universities of different research capabilities in the field of dairy technology, more industries with suitable food vehicles for LVWPI fortification that may cover wider socio-economic spectrum in the Philippine population, and more government agencies, such as the DOH, Department of Science and Technology (DOST), the National Economic Development Authority (NEDA), and the Institute of Small-Scale Industries (ISSI).

5.9. CONCLUSION

The proposed model may prove effective in bringing forth the LVWPI production technology from the USA to the Philippines where the intended recipient may take advantage of the health benefits and functional properties of native whey proteins while improving productivity in the dairy sector and effecting positive impact to the dairy industry's economic status. The model presents future potentials in sustaining the positive impact of the technology and providing opportunities for coordinated efforts in branching out to other related technologies apt to Philippine setting.

5.10 ACKNOWLEDGMENT

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APPENDIX A

Development of flux model for the ultrafiltration of virgin whey

Merin and Cheryan (1980) proposed the following flux model for the ultrafiltration of cheese whey:

$$J = J_o V^{-b} \qquad \text{(A.1)}$$

But, $J = \frac{1}{A}\frac{dV}{dt}$

$$\frac{1}{A}\frac{dV}{dt} = J_o V^{-b} \qquad \text{(A.2)}$$

$$\frac{dV}{dt} = AJ_o V^{-b}$$

$$\frac{dV}{V^{-b}} = AJ_o dt$$

or $V^b\, dV = A\, J_o\, dt$

Integrating,

$$\int V^b dV = J_o A \int dt$$

$$\frac{1}{b+1} V^{b+1} = J_o A t$$

$$V^{b+1} = J_o A (b+1) t$$

$$V = \left[J_o A (b+1)\right]^{\frac{1}{b+1}} t^{\frac{1}{b+1}}$$

Define: $k = \left[J_o A(b+1)\right]^{\frac{1}{b+1}}$ (A.4)

Then $V = kt^{\frac{1}{b+1}}$ (A.3)

Taking the differential of equation (A.3):

$$\frac{dV}{dt} = \frac{\left[J_o A(b+1)\right]^{\frac{1}{b+1}}}{b+1} t^{\frac{-b}{b+1}}$$

or $$\frac{dV}{dt} = k\, t^{\frac{-b}{b+1}}$$

But, $$\frac{1}{A}\frac{dV}{dt} = J$$

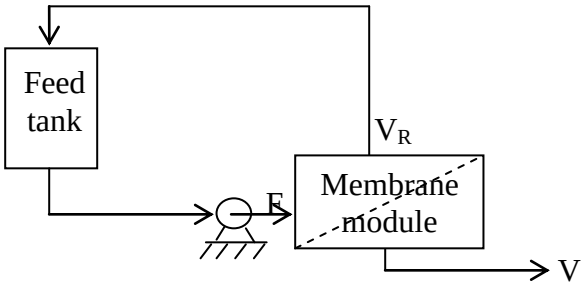
Therefore, $$J = \frac{k}{A(b+1)} t^{\frac{-b}{b+1}}$$ (A.5)

In the UF of VW, it has been hypothesized that since the composition of VW is constant and that it contains native whey proteins, long-term fouling will be uniform regardless of feed quantity, F, and that short-term fouling will be governed by F/A. Since F/A is directly related to the design of the UF system, then a flux equation that expresses J in terms of F/A will be useful in UF system design and optimization.

The following shows how equation A.5 was modified to express J in terms of F/A and the long-term fouling parameter, b.

A. Solute Balance:

Simplified UF system schematic:



At any time, t, in the membrane module:

$$V_R = F - V \tag{A.6}$$

Solute (protein) balance around the module:

$$-\frac{d(V_R C)}{dt} = JAC(1-R) \tag{A.7}$$

where: $R = \text{rejection coefficient} = \frac{C - C_P}{C_o} = 1 - \frac{C_P}{C_o}$

In equation (A.7):

$$-\frac{d(V_R C)}{dt} = \left[\frac{k}{A(b+1)} t^{\frac{-b}{b+1}}\right] AC(1-R)$$

or, $$-d(V_R C) = AC(1-R)\left[\frac{k}{A(b+1)} t^{\frac{-b}{b+1}}\right] dt \quad \div \quad V_R C$$

$$-\frac{d(V_R C)}{V_R C} = \frac{A(1-R)\left[\frac{k}{A(b+1)} t^{\frac{-b}{b+1}}\right] dt}{V_R}$$

$$-\frac{d(V_R C)}{V_R C} = \frac{A(1-R)\left[\frac{k}{A(b+1)} t^{\frac{-b}{b+1}}\right] dt}{V_R}$$

But $V_R = F - V$ and $V = k\, t^{\frac{1}{b+1}}$

Therefore, $$-\frac{d(V_R C)}{V_R C} = \frac{A(1-R)\left[\frac{k}{A(b+1)} t^{\frac{-b}{b+1}}\right] dt}{F - kt^{\frac{1}{b+1}}} \times \frac{F}{F}$$

$$-\frac{d(V_R C)}{V_R C} = \frac{\frac{A}{F}(1-R)\left[\frac{k}{A(b+1)} t^{\frac{-b}{b+1}}\right]}{1-\frac{k}{F} t^{\frac{1}{b+1}}} dt$$

$$\int_{FC_o}^{V_R C} \frac{d(V_R C)}{V_R C} = -\frac{A}{F}(1-R)\int_0^t \frac{\frac{k}{A(b+1)} t^{\frac{-b}{b+1}}}{1-\frac{k}{F} t^{\frac{1}{b+1}}} dt$$

$$\ln(VC)\Big|_{F,C_o}^{V_R,C} = (1-R)\ln\left(1-\frac{k}{F} t^{\frac{1}{b+1}}\right)\Big|_0^t$$

$$\ln\frac{V_R C}{FC_o} = (1-R)\left\{\ln\left(1-\frac{k}{F} t^{\frac{1}{b+1}}\right) - \ln(1-0)\right\} \to 0$$

$$\ln\frac{V_R C}{FC_o} = (1-R)\left[\ln\left(1-\frac{k}{F} t^{\frac{1}{b+1}}\right)\right]$$

or, $$\frac{V_R C}{F C_o} = \left[1 - \frac{k}{F} t^{\frac{1}{b+1}}\right]^{(1-R)} \quad \text{but} \quad k = \left[J_o (b+1)\right]^{\frac{1}{b+1}}$$

Therefore,

$$\frac{V_R C}{F C_o} = \left\{1 - \frac{\left[J_o A (b+1)^{\frac{1}{b+1}}\right]}{F} t^{\frac{1}{b+1}}\right\}^{(1-R)} \qquad \text{(A.8)}$$

To solve for relative concentration, $\frac{C}{C_o}$:

$$\frac{V_R C}{F C_o} = \left[1 - \frac{k}{F} t^{\frac{1}{b+1}}\right]^{(1-R)}$$

But $V = k\, t^{\frac{1}{b+1}}$ and $V_R = F - V$

Therefore, $V_R = F - k t^{\frac{1}{b+1}}$

Substituting in equation (A.8):

$$\frac{\left(F - k t^{\frac{1}{b+1}}\right) C}{F C_o} = \left[1 - \frac{k}{F} t^{\frac{1}{b+1}}\right]^{(1-R)}$$

Solving for $\frac{C}{C_o}$:

$$\frac{C}{C_o} = \left[1 - \frac{k}{F} t^{\frac{1}{b+1}}\right]^{(1-R)} \bullet \frac{F}{F - k\, t^{\frac{1}{b+1}}}$$

$$\frac{C}{C_o} = \left[1 - \frac{k}{F} t^{\frac{1}{b+1}}\right]^{(1-R)} \bullet \frac{F/F}{\left(F - k\, t^{\frac{1}{b+1}}\right)/F}$$

$$\frac{C}{C_o} = \left[1 - \frac{k}{F} t^{\frac{1}{b+1}}\right]^{(1-R)} \bullet \frac{1}{1 - \frac{k}{F} t^{\frac{1}{b+1}}}$$

Or: $$\frac{C}{C_o} = \left[1 - \frac{k}{F} t^{\frac{1}{b+1}}\right]^{(1-R)} \bullet \left(1 - \frac{k}{F} t^{\frac{1}{b+1}}\right)^{-1}$$

Therefore: $$\frac{C}{C_o} = \left[1 - \frac{k}{F} t^{\frac{1}{b+1}}\right]^{-R}$$

or, $$\frac{C}{C_o} = \left[1 - \frac{\left[J_o\, A\,(b+1)\right]^{\frac{1}{b+1}}}{F}\; t^{\frac{1}{b+1}}\right]^{-R}$$ (A.9)

B. Process time, t:

Taking ln of both sides of equation (A.9):

$$\ln\frac{C}{C_o} = -R\left[1-\frac{k}{F}t^{\frac{1}{b+1}}\right]$$

$$\frac{1}{R}\ln\left(\frac{C_o}{C}\right) = \ln\left[1-\frac{k}{F}t^{\frac{1}{b+1}}\right]$$

$$\left(\frac{C_o}{C}\right)^{\frac{1}{R}} = 1-\frac{k}{F}t^{\frac{1}{b+1}}$$

Solving for t:

$$t^{\frac{1}{b+1}} = \left[1-\left(\frac{C_o}{C}\right)^{\frac{1}{R}}\right]\left(\frac{F}{k}\right)$$

$$t = \left\{\left[1-\left(\frac{C_o}{C}\right)^{\frac{1}{R}}\right]\left(\frac{F}{k}\right)\right\}^{(b+1)} \qquad \text{(A.10)}$$

C. Flux in terms of F/A:

Note that:

$$C = C_o\,(CF) \qquad \text{(A.11)}$$

or $$\frac{C}{C_o} = C_o\,(CF)^R$$

But $$\frac{C}{C_o} = \left[1-\frac{k}{F}t^{\frac{1}{b+1}}\right]^{-R} \qquad \text{(A.12)}$$

and $$J = \frac{k}{A(b+1)} t^{\frac{-b}{b+1}}$$

$$= \frac{k}{A(b+1)} t^{-1} t^{\frac{1}{b+1}}$$

$$= \frac{k}{A(b+1)t} t^{\frac{1}{b+1}}$$

Therefore, $$JA(b+1)t = k\, t^{\frac{1}{b+1}}$$

Solving equations (A.8) and equation (A.6) simultaneously:

$$\frac{C}{C_o} = \left[1 - \frac{JA(b+1)t}{F}\right]^{-R}$$

Therefore, $$(CF)^R = \left[1 - \frac{JA(b+1)t}{F}\right]^{-R}$$

or, $$(CF)^{-1} = 1 - \frac{JA(b+1)t}{F}$$

$$\frac{JA(b+1)t}{F} = 1 - \frac{1}{CF}$$

$$J = \frac{F}{A}\left(\frac{1}{b+1}\right)\left(1 - \frac{1}{CF}\right) t^{-1}$$

or, $$J = \frac{F}{A}\left(\frac{1 - \frac{1}{CF}}{b+1}\right)\left(\frac{1}{t}\right)$$

or, $$J = \frac{F}{A}\left(\frac{1}{b+1}\right)\left(\frac{1-\frac{1}{CF}}{t}\right) \qquad \text{(A.13)}$$

where: $\frac{F}{A}$ = initial fouling parameter

$\frac{1-\frac{1}{CF}}{b+1}$ = long-term fouling parameter

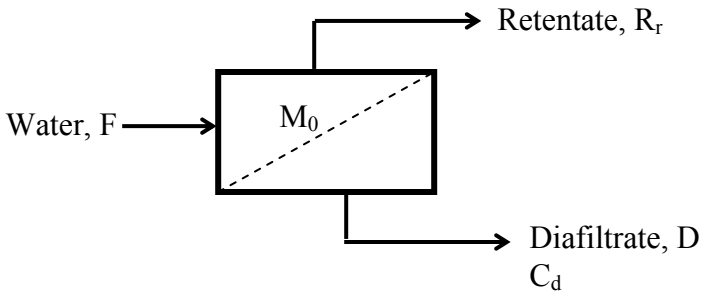
APPENDIX B

Estimation of diafiltration time, total diavolume (or diamass) and UF retentate composition

Diafiltration is an important step in the concentration of whey proteins (WP) in that it increases the purity of the proteins in the final retentate. This step involves the continuous addition of water of equal amount as the permeate while filtration proceeds to maintain the amount of the retentate in the membrane system, allowing a more extensive removal of lactose, minerals and small peptides. The amount of diavolumes (or diamass) to be used and the diafiltration time depend on the desired purity of the WP in the retentate.

A. Working equation for the determination of the amount of diafiltrate needed

Membrane module schematic:



Lactose balance around the membrane module:

$$M_0 C = M_0 (C + dC) + J_f C_P dt \qquad \text{(B.1)}$$

where: M_0 = mass of retentate before diafiltration

C = concentration of lactose at anytime, t

C_d = concentration of lactose in the permeate

J_d = diafiltrate flow rate

Simplifying equation (B.1):

$$-M_0 dC = J_d C_d dt \qquad \text{(B.2)}$$

For the case where rejection, R, is equal to zero:

$$C = C_d$$

Then equation (B.2) becomes

$$-M_0 dC = J_d C dt$$

or, $$-M_0 \frac{dC}{C} = J_d dt$$

Integrating,

$$-M_0 \int_{C_0}^{C} \frac{dC}{C} = J_d \int_0^t dt$$

$$-M_0 \ln \frac{C}{C_0} = J_d t$$

$$\ln \frac{C_0}{C} = \frac{J_d t}{M_0} \qquad \text{(B.3)}$$

But $J_d t = D$

then $$\ln \frac{C_0}{C} = \frac{D}{M_0} \qquad \text{(B.4)}$$

Note that $\frac{D}{M_0}$ = the number of diavolumes (or "diamass").

For the case where R ≠ 0:

$$C_d = C\,(1 - R)$$

Therefore, equation (B.4) becomes

$$\ln\frac{C_0}{C} = (1-R)\frac{D}{M_0}$$

Solving for the mass of diafiltrate needed, D:

$$D = \frac{M_0}{1-R}\left(\ln\frac{C_0}{C}\right) \qquad \text{(B.5)}$$

B. Sample Calculations

i. Estimate the values of C_0 and C.

Note: Entries in red font are input data, and entries in blue font are calculated numbers based on input data.

Step 1: Provide the following information (Input data from preliminary runs):

Input data:

Amount of whey to be processed = 1,050 kg

Composition of the whey stream to be ultrafiltered:

% WP = 0.34
% Lactose = 4.5
% Salt = 1.0
% Water = 94.16

} Values from preliminary runs

Retention of lactose = 0.1

Desired WP mass concentration factor (MCF) in the UF = 12

Desired fraction of lactose after diafiltration = 0.1

Step 2: Calculate C_0 in the first-stage ultrafiltration step

Composition of the water phase before ultrafiltration:

$$\%\ \text{Lactose} = \left(\frac{4.5}{4.5+1.0+94.16}\right)\times 100 = 4.52$$

$$\%\ \text{Salt} = \left(\frac{1.0}{4.5+1.0+94.16}\right)\times 100 = 1.00$$

$$\%\text{Water} = \left(\frac{94.16}{4.5+1.0+94.16}\right)\times 100 = 94.48$$

Concentration of lactose in the final concentrate, C_0

$$= (\%\ \text{Lactose})(\text{MCF})^{R}$$

$$= (4.52)(12)^{0.1} = 5.80$$

$$\text{Final mass of concentrate} = \frac{1{,}050}{12} = 87.5\ \text{kg}$$

Mass of water phase in the concentrate

$$= \text{final mass of concentrate} - \text{mass of WP}$$

$$= (87.5\ \text{kg}) - (1{,}050\ \text{kg})(0.0034) = 83.93\ \text{kg}$$

Mass composition of the final concentrate:

WP = (1,050 kg)(0.0034) = 3.57 kg

Lactose = (87.5 kg)(0.0058) = 4.86 kg

Salt = (83.93 kg)(0.01) = 0.84 kg

Water = (87.5 – 3.57 – 4.86 – 0.84) kg = 78.23 kg

% composition (w/w) of the final concentrate:

WP = 4.08

Salt = 1.06

Lactose = 5.79

Water = 95.04

$$\%\text{WP (dry basis)} = \left(\frac{3.57}{3.57 + 4.86 + 0.84}\right) \times 100 = 38.51$$

ii. Estimate the amount of diafiltrate and the concentration of WP in the diafiltered retentate

Using equation B.5, the mass of diafiltrate (water) needed:

$$D = \frac{M_0}{1 - R}\left(\ln \frac{C_0}{C}\right) = \frac{78.22}{1 - 0.1}\left(\ln \frac{5.79}{0.1}\right) = 352.74 \text{ kg}$$

Mass composition of the final concentrate:

WP = 3.57 kg

Lactose = (0.001) (83.93 kg) = 0.08393 kg

Salt = 0.84 kg

Water = 78.23 kg

% composition (w/w) of the final concentrate:

$$\%\ \text{WP} = \frac{3.57}{87.5} \times 100 = 4.08$$

% Lactose = 0.10

$$\%\ \text{Salt} = \frac{0.84}{87.5} \times 100 = 0.96$$

% Water = 100 – 4.08 – 0.1 – 0.96 = 94.86

% WP in the diafiltered retentate (dry basis)

$$= \frac{4.08}{4.08 + 0.10 + 0.96} \times 100 = 79.38$$

C. Estimation of diafiltration time based on flux history data:

Measured permeate flux at the chosen MCF = 13.24 $\frac{kg}{hr - m^2}$

Filtration time to reach 13x in the UF, $t_{ultrafiltration}$ = 9 hrs

Diafiltration time, $t_{ultrafiltration} = \frac{352.74\,kg}{13.24\,\frac{kg}{hr - m^2} \bullet 5.9\,m^2} = 4.52$ hrs

Total process time, $t_{ultrafiltration} + t_{diafiltration}$ = 13.52 hrs