UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS♦

JUDUL: COMPARATIVE STUDY ON MILK PROTEIN SEPARATION USING DIFFERENT TECHNIQUE

SESI PENGAJIAN : 2008/2009

Saya **WAN NURFARAH BINTI WAN ALI**

(HURUF BESAR)

mengaku membenarkan tesis (PSM/~~Sarjana~~/~~Doktor Falsafah~~)* ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut :

1. Tesis adalah hakmilik Universiti Malaysia Pahang.
2. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (√)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☑	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS) (TANDATANGAN PENYELIA)

Alamat Tetap:
No. 18, Lorong 16,
Balok Baru Fasa II,
26100 Kuantan, Pahang.

Shalyda binti Md Shaarani @ Md Nawi
Nama Penyelia

Tarikh: **APRIL 2009** Tarikh: **APRIL 2009**

CATATAN :
* Potong yang tidak berkenaan.
** Jika tesis ini **SULIT** atau **TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasiberkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai **SULIT** atau **TERHAD**.
♦ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Lapuran Projek Sarjana Muda (PSM).

"I hereby declare that I have read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the award of the degree of
Bachelor of Chemical Engineering
(Biotechnology)"

Signature : .....
Supervisor : Shalyda binti Md Shaarani @ Md Nawi
Date : April 2009

A COMPARATIVE STUDY ON MILK PROTEIN SEPARATION USING DIFFERENT TECHNIQUE

WAN NURFARAH BINTI WAN ALI

A thesis submitted in fulfillment of the
requirements for the award of the degree of
Bachelor of Chemical Engineering
(Biotechnology)

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

APRIL 2009

DECLARATION

I declare that this thesis entitled “A Comparative Study on Milk Protein Separation Using Different Technique” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ……

Name of Candidate : Wan Nurfarah binti Wan Ali

Date : April, 2009

Special Dedication of This Grateful Feeling to My…

Beloved Mother and Father,
My family members that always love me,
My friends, my fellow colleague,
Supportive Lecturer
Mrs. Shalyda binti Md Shaarani @ Md Nawi,
and all faculty members.

For all your care, support and believed in me.

ACKNOWLEDGEMENT

In the name of Allah, The Most Gracious, The Most Merciful. Praise to Allah S.W.T by whose grace and blessing I receive guidance in completing this study. Thanks for His greatest love and blessing.

In this opportunity, I would like to express my appreciation to all people who involved directly or not directly in helping me to complete this thesis. There are many people who involved in and pay contribution for this thesis. The people who gave me guidance and share their times and experiences to give me full understanding and moral support to complete my thesis, I would like to express my special thanks to Mrs. Shalyda binti Md Shaarani @ Md Nawi for her will to share her experience, encouragement, guidance, critics and assistance to help me fulfill my thesis as a supervisor. Also thanks to Mr. Zainal bin Giman and Mr Mohd Anuar bin Hj. Ramli for gave me full support in term of preparation the lab equipment and material of my research.

In this opportunity, I would like to thank all lecturers and staffs in Faculty of Chemical and Natural Resource Engineering University Malaysia Pahang especially teaching engineers who spend their time to give a space for completing this research. Indeed, I indebted all staffs and lectures for their endless support and encouragements.

In particular, my sincere thankful is also extend to my family, all my colleagues and others who have provide assistance at various. May Allah bless us. Wassalam.

ABSTRACT

Fractionation of full cream milk protein using two protein separation techniques which are crossflow filtration and conventional method was examined. Two membrane of MWCO of 10 and 50 kDa of polyethersulfone material were used to determine the efficiency of the process for crossflow filtration and filter paper were used for conventional method. The performance of crossflow filtration was determined under various processing conditions that include the transmembrane pressure across the membrane and the concentrations of milk sample from permeate. Among the membrane pore sizes tested, it was found that the 10 kDa MWCO membrane cassette can collected more protein compare with 50 kDa MWCO membrane cassette and conventional method. Permeate flux was primarily affected by pump speed and transmembrane pressure. The optimal operating conditions for this separation were transmembrane pressure of 15 psi for 10 kDa MWCO membrane. From the experiment, it showed that the permeate flux increased by increasing the time of operation, and transmembrane pressure. The high of transmembrane pressure can affect the process of protein separation because some fouling occurred at the membrane.

ABSTRAK

Pecahan protein dalam susu penuh krim dengan menggunakan teknik mengasingkan protein iaitu penapisan aliran halangan dan cara mengikut kebiasaan.diselidik. Dua membran yang digunakan iaitu 10 dan 50 kDa jisim pemotongan molekul yang diperbuat daripada ‘polyethersulfone’ telah digunakan untuk menentukan keberkesanan proses penapisan menggunakan penapis aliran halangan dan kertas turas menggunakan cara kebiasaan. Perlaksanaan bagi penapis aliran halangan boleh ditentukan dengan beberapa keadaan proses yang terdiri daripada tekanan transmembran yang melalui membran dan kepekatan sampel susu daripada ‘permeate’. Antara ujian saiz liang membran, dijumpai bahawa membran kaset 10 kDa jisim pemotongan molekul boleh mengumpul lebih banyak protein berbanding membran keset 50 kDa jisim pemotongan molekul dan cara kebiasaan. Halaju pum dan tekanan transmembran boleh memberi kesan terhadap ‘permeate’ fluks. Keadaan operasi yang optimal bagi perpisahan protein ini ialah pada tekanan transmembran 15 psi untuk membran 10 kDa jisim pemotongan molekul. Melalui ujikaji ini, menunjukkan bahawa ‘permeate’ fluxs meningkat dengan peningkatan masa operasi dan peningkatan tekanan transmembran. Tekanan transmembran yang tinggi memberi kesan terhadap proses perpisahan protein kerana membran telah dicemari oleh bahan yang tidak dikehendaki.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF SYMBOLS

BSA	-	bovine serum albumin
CF	-	concentration factor
CFF	-	crossflow filtration
DNA	-	deoxyribonucleic acid
Jv	-	permeate flux
kDa	-	kilo Dalton
MF	-	microfiltration
MWCO	-	molecular weight cutoff
NF	-	nanofiltration
NPN	-	non-protein
OD	-	optical density
PES	-	polyethersulfone
RNA	-	ribonucleic acid
RO	-	reverse osmosis
SCU	-	self-contained unit
TMP	-	transmembrane pressure
U	-	cross-flow velocity
UF	-	ultrafiltration
WPC	-	whey protein concentration
ΔP	-	transmembrane pressure

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Protein is one of the nutrients. Some foods contain much higher amounts of specific nutrients than others. It is important to realize that all goods contain more than one nutrient and most foods contain substantial amounts of several nutrients. Protein is an essential nutrient. Protein is contained in every part of our body, the skin, muscles, hair, blood, body organs, eyes, even fingernails and bone.

Proteins play a major role in ensuring our health well being. There are so many functions of proteins in the body. The primary functions of proteins include building and repairing of body tissues, regulation of body processes and formation of enzymes and hormones. Our body requires proteins for the purpose of maintenance and healthy growth. The need for consuming proteins is especially more for infants, young children, pregnant women and recovering patients.

One example of food that contains protein is milk. Milk does not contain a large quantity of proteins, but it is one of the most excellent sources of protein that have a good quality of protein content. Protein in milk contains all the essential amino acids that the body requires. Milk proteins have different functional properties such as solubility, foaming, whipping, emulsification, gelation and flavor entrapment ability (Punidadas P. *et al.*, 1999).

Milk protein can be separated by using a membrane processes. Membrane processing is a technique that permits concentration and separation without the use of heat. Particles are separated based on their molecular size and shape with the use of pressure and specially designed semi-permeable membranes. There are many types of membrane processing. The commonly used in industry is micro filtration, ultra filtration, reverses osmosis and electro dialysis.

Cross-flow membrane systems are one of the efficient membrane separation methods for protein separation. In cross-flow filtration, the feed is passed across the filter membrane at some pressure difference. Material which is smaller than the membrane pore size passes through the membrane as permeate or filtrate, and everything else is retained on the feed side of the membrane as retentate.

Another technique for protein separation is using normal isolation. In this method, isoelectric precipitation is used for the isolation of casein from milk.

1.2 Problem Statement

There are several methods that we can use for protein fractionation today. It can give different quality of protein produced from there. So, the study of comparative protein separation technique is important because from that technique, it will show the effectiveness of the membrane system to separate proteins. The best one of the methods can be choose. Other than that, we can reduce operating cost because before that, the conventional method in industry is difficult to scale up, complex and used expensive equipment.

1.3 Research Objectives

This research was conducted to achieve several objectives. One of the most important objectives is to determine the best method of protein separation by using three types of protein separation (ultrafiltration (UF), microfiltration (MF), and isolation. Then the next objective is to increase the percentage of milk protein recovered using protein separation system. The last objective will be comparison the quantity of protein in deferent types of milk.

1.4 Research Scope

The first scope for this work is to find the most effective techniques for protein separation based on the crossflow rate, transmembrane pressure and permeate flow. The transmembrane pressure can be define as a difference in pressure between the filtrate side of the membrane and the permeate side of the membrane. The second scope of this work is to determine the best method to produce high quantity of milk protein and the last scope is to study the different quantity of milk protein in the various types of milk.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Milk is produced by the mammary glands of female mammals. It provides the primary source of nutrition for newborn mammals before they are able to digest other types of food. The composition of milk differs widely between species. Factors such as the type of protein, the levels of various vitamins and minerals, and the size of the bufferfat globules and the strength of the curd are among those than can vary.

2.1.1 Structure of Protein

Proteins are organic compounds containing of small units. These units are the amino acids or we can call it building blocks of protein. As we know that there are 20 different amino acids which are commonly known. Each different protein is composed of various amino acids put together with almost limitless combinations. Most proteins are large molecules that may contain several hundred amino acids arranged in branches and chains. Proteins are chains of amino acid molecules connected by peptide bonds.

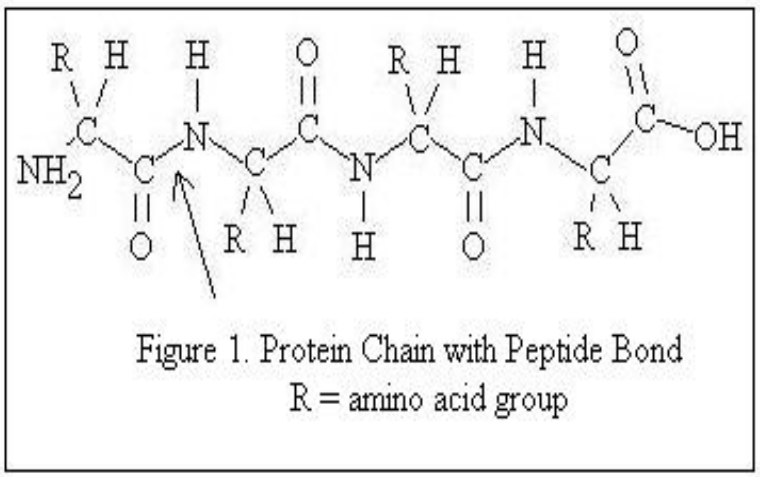

Figure 2.1: Protein Chain with Peptide Bond

The structure of protein divided into four, which are primary structure, secondary structure, tertiary structure, and quaternary structure. The primary structure of proteins consists of a polypeptide chain of amino acids residues joined together by peptide linkages. Amino acids contain both a weakly basic amino group, and weakly acid carboxyl group both connected to a hydrocarbon chain. The three dimensional organization of proteins, also involves secondary, tertiary, and quaternary structures. The secondary structure refers to spatial arrangement of amino acid residues that are near one another in the linear sequence. The alpha-helix and β-pleated sheat are examples of secondary structures arising from regular and periodic steric relationships.

The tertiary structure refers to the spatial arrangement of amino acid residues that are far apart in the linear sequence, giving rise to further coiling and folding. If the protein is tightly coiling and folded into a somewhat spherical shape, it is called a globular protein. If the protein consists of long polypeptide chains which are intermolecularly linked, they are called fibrous proteins. Quanternary structure occurs when proteins with two or more polypeptide chain subunits are associated.

Milk contains 3.3% total protein. There are 9 essential amino acids in milk proteins required by humans. Milk proteins are synthesized in the mammary gland, but 60% of the amino acids used to build the proteins are obtained from the cow's diet. Total milk protein content and amino acid composition varies with cow breed and individual animal genetics.

There are 2 major categories of milk protein that are broadly defined by their chemical composition and physical properties. The casein family contains phosphorus and will coagulate or precipitate at pH 4.6. The serum (whey) proteins do not contain phosphorus, and these proteins remain in solution in milk at pH 4.6. The principle of coagulation, or curd formation, at reduced pH is the basis for cheese curd formation. In cow's milk, approximately 82% of milk protein is casein and the remaining 18% is serum, or whey protein.

2.1.2 Milk Protein Fractionation

The nitrogen content of milk is distributed among caseins (76%), whey proteins (18%), and non-protein (NPN) (6%). This does not include the minor proteins that are associated with the FGM. This nitrogen distribution can be determined by the Rowland fractionation methods, which is precipitation at pH 4.6 to separates caseins from whey nitrogen and precipitation with sodium acetate and acetic acid (pH 5.0) to separates total proteins from whey NPN.

The concentration of proteins in milk is given in the table 2.1:

Table 2.1: Concentration of Protein in Milk

	grams/ litre	**% of total protein**
Total Protein	33	100
Total Caseins	26	79.5
Alpha s1	10	30.6
Alpha s2	2.6	8.0
Beta	9.3	28.4
Kappa	3.3	10.1
Total Whey Proteins	6.3	19.3
Alpha lactalbumin	1.2	3.7
Beta lactoglobulin	3.2	9.8
BSA	0.4	1.2
Immunoglobulins	0.7	2.1
Proteose peptone	0.8	2.4

Caseins, as well as their structural form casein micelles, whey proteins, and milk enzymes will now be examined in further detail.

2.1.3 Quality of Milk Protein

The protein in milk has a quality higher than many other foods but the quantity of milk protein is low due to high water content. There are all the essential amino acids in the milk protein. These amino acids are required by the body for optimum growth, for this reason more of the protein can be used for protein anabolism so there's less chance the protein in milk will be converted to fat and store.

Protein can be used for building parts of the human body. It can be determined by the type and amounts of amino acids present in the particular protein

molecule. The body has the ability to interconvert and make some of the amino acids. However, not all the amino acids can put together in the body but only eight of the amino acids. It is must be supplied by food we eat. These eight amino acids are called essential amino acids. The value of proteins can be determined by the presence in adequate amounts of the eight essential amino acids. The protein of cereals, most beans, and vegetables may contain all the essential amino acids, but the amounts in these foods are less than ideal.

2.1.4 Protein Requirement

The amount of protein needed varies for different age groups, size and growth stage. Protein is required for maintaining body tissues for an adult that is in the level to achieved maximum growth. Periods of growth, including infancy, childhood and pregnancy, increase the protein need to provide building materials. Other than that, the physiological states such as injury, surgery, or burns, increase the need for protein to provide repairing materials. The following table gives the recommended dietary allowances for different group, established by the Food and Nutrition Board, National Academy of Sciences, National Research Council. 1980 Revised (Georgia C. Lauritzen, 1992).

Table 2.2: The recommended dietary allowances for different group

Amount of Protein Needed Daily (Grams)					
Children		**Women**		**Man**	
Age	Gram	Age	Gram	Age	Gram
1-3	23	11-14	46	11-14	45
4-6	30	15-18	46	15-18	56
7-10	34	19-22	44	19-22	56
		23+	44	23+	56
		Pregnant	+30		
		Lactating	+20		

Protein yields approximately 4 calories per gram, which is the same energy concentration as carbohydrate. The recommended level of protein intake for the general population is 12 to 15 percent of total calories. Therefore, someone consuming 2,000 calories per day has an energy equivalent of 240 to 300 calories (60 to 75 grams) of protein per day.

2.1.5 Food Source

Protein is available from both animal and plant sources. The typical U.S. diet is a mixture of protein sources (Georgia C. Lauritzen, 1992). Variety in choices will provide an adequate diet. The following table shows protein content in some typical foods.

Table 2.3: Protein Content in Some Typical Foods

Food	Amount	Protein in Gram
Chicken	3 oz.	20
Ground Beef	3 oz.	21
Pork Chop, lean	2 oz.	15
Milk non-fat (skim)	**1 cup**	**8.35**
Egg	1	6
Cheddar Cheese	1 oz.	7
Beans	3/4 cup	11

Milk definitely serves as one of the most excellent sources of proteins and this can be attributed to its rich quality protein content. Protein in milk contains all the essential amino acids that the body requires. About half the milk produced in the United States is sold as fluid milk and cream. Much of the rest comes to the market as butter, cheese, and ice cream. The available market forms of milk include fluid milk - whole, reduced fat (2 percent), low fat (1 percent), fat-free (non fat), chocolate, and many others like dry milk, cream, and cultured milk products such as

yogurt and buttermilk. Here is presented milk proteins table that depicts the protein content of milk.

Table 2.4: Protein Content of Milk

Milk (1 cup)	Protein (g)
Milk, reduced fat (2 percent)	8.13g
Milk, low fat (1 percent)	8.03g
Milk, non-fat (skim)	8.35g
Milk, evaporated, non fat, canned	19.33g
Milk, evaporated, condensed	24.2g
Milk, buttermilk	8.11g
Milk, chocolate, regular	7.93g
Milk, soy	6.74g

2.2 Membrane Separation

Proteins are very diverse. They differ by size, shape, change, hydrophobicity, and their affinity for other molecules. All these properties can be exploited to separate them from one another so that they can be studied individually (Alberts, Bray *et al*., 1998)

2.2.1 Principle of Membrane Separation

There are many types of membrane separation. That is reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), and microfiltration (MF) process. The membrane separations are used in the dairy industry and have a different purposes:

RO – used for dehydration of whey, UF permeate and condensate.
NF – used when partial desalination of whey, UF permeates or retentate is required.

UF – typically used for concentration of milk proteins in milk and whey and for protein standardization of milk intended for cheese, yoghurt and some other products.

MF – basically used for reduction of bacteria in skim milk whey and brine, but also for defecting whey intended for whey protein concentration (WPC) and for protein fractionation.

The general flow patterns of the various membrane separation systems are illustrated in figure 2.2.

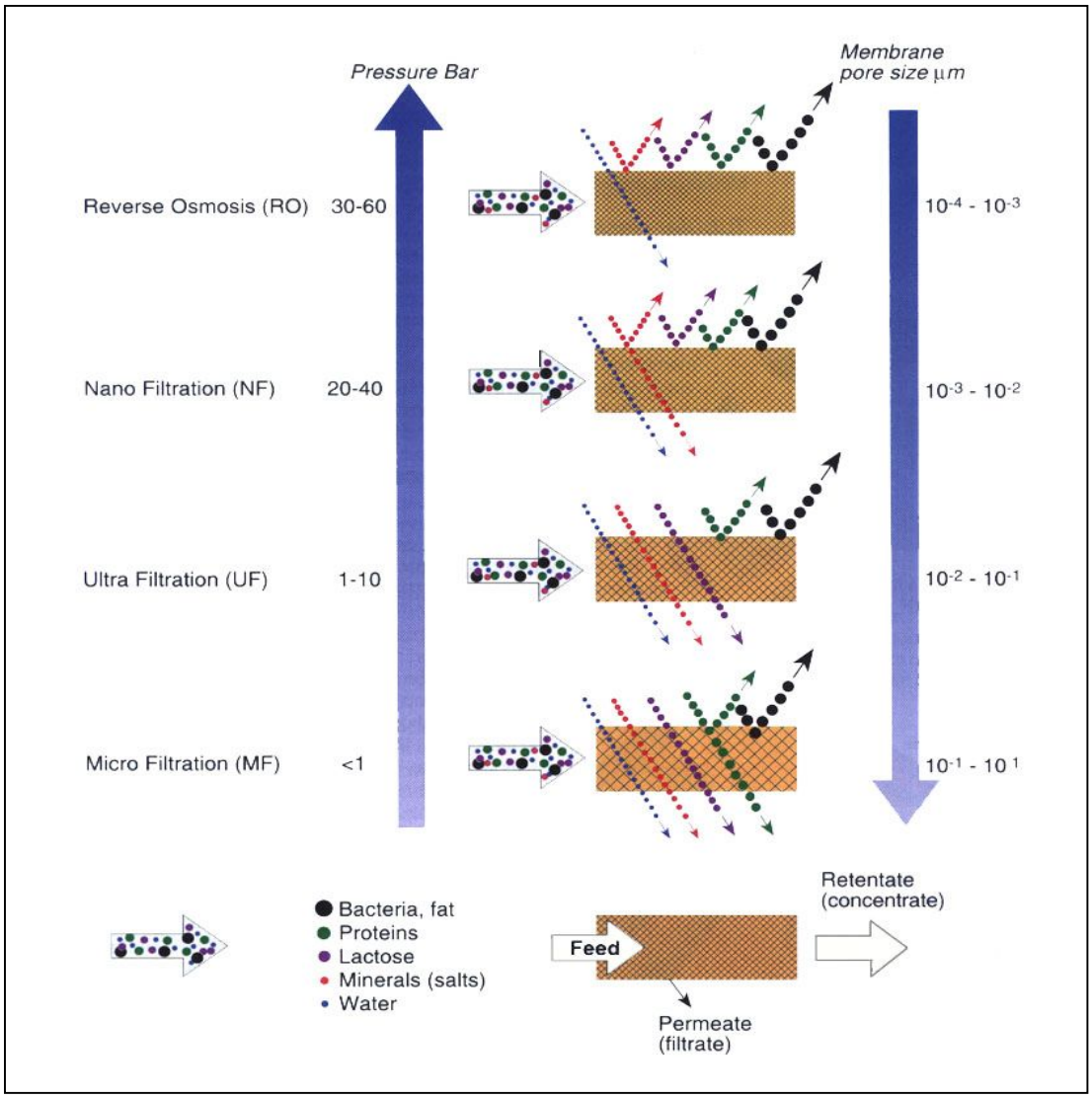

Figure 2.2: Principle of Membrane Separation

2.2.2 Crossflow Filtration

The most commonly used of protein separation in industrial membrane filtration is crossflow or tangential flow filtration. The solution to be filtered is flowed through the surface of the membrane. The materials that pass across the membrane are referred to as the filtrate. The materials which do not pass through the membrane are referred to as the retentate. This retentate is recycle back to the feed reservoir to be filtered. There is shear rate in the crossflow filtration. This shear rate is essentially the velocity at which the filtrate is passed across the surface of the membrane. The function of the shear rate is to remove any particles which may have collected at the surface of the membrane. It is also maintain a relatively steady flux across the membrane.

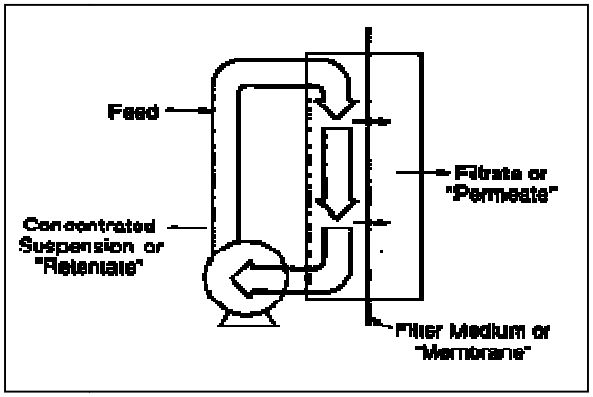

Figure 2.3: Cross-flow Filtration

Crossflow filtration, CFF can be used to fractionate milk proteins. In CFF, as liquid products flow parallel to the surface of the membrane, the permeate pass through the membrane. The permeate flux depends on the properties of the membrane, product and the operational conditions (transmembrane pressure, cross-flow velocity, concentration factor, running time, and temperature) (Punidadas and Rizvi., 1998).

2.2.2.1 Protein Separation by Ultrafiltration

Protein separation is rapidly becoming more selective through improvements in membrane. Membrane separation techniques offer advantages of lower cost and ease to scale up for commercial production. However, the lack of membrane selectivity and its fouling due to protein absorption during filtration has severely restricted Ultrafiltration (UF) applications. Now, UF has been widely used as preferred method for protein concentration and buffer exchange, and replaced size exclusion chromatography in these applications (Arunima Saxena et al., 2008).

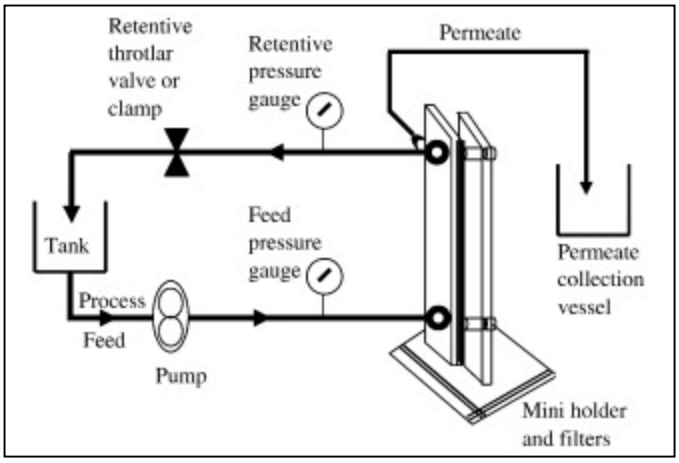

Figure 2.4: A schematic diagram for the ultrafiltration membrane set-up

Ultrafiltration (UF) membranes can retain macromolecular solutes. Solute retention is determined by solute size. UF separates solutes in the molecular weight range of 5 kDa to 500 kDa. UF membranes have pores ranging from 1 to 20 nm in diameter (Raja Ghosh, 2003). In UF, the membrane pore size is larger allowing some components to pass through the pores with the water. It is a separation/ fractionation process using a 10,000 MW cutoff, 40 psig, and temperatures of 50-60°C with polysulfone membranes. The permeate contains low-molecular-weight organic solutes and salts. UF is widely used in the fractionation of milk and whey, and also finds application in protein fractionation.

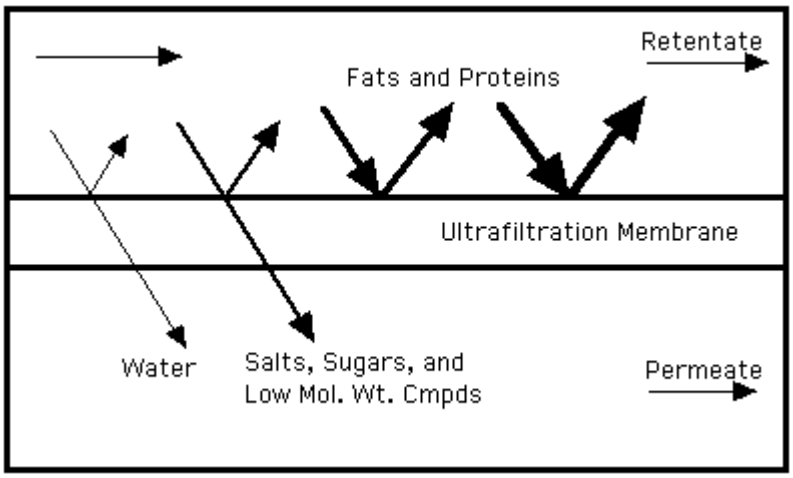

Figure 2.5: Ultrafiltration

2.2.2.2 Protein Separation by Microfiltration

Microfiltration (MF) widely used for the separation, purification and clarifying of protein containing solutions. The macromolecules and proteins involved in these processes are much smaller in size than the pores of the MF membrane and should not normally be retained by the membranes. The basic operational concept of MF leads to a solute concentration that is higher and close to the membrane surface than it is in the bulk feed stream. There are two standard modes of operation in MF. That is dead end and cross flow configurations. In the crossflow mode, the fluid to be filtered flows parallel to the membrane surface and permeate through the membrane due to pressure difference.

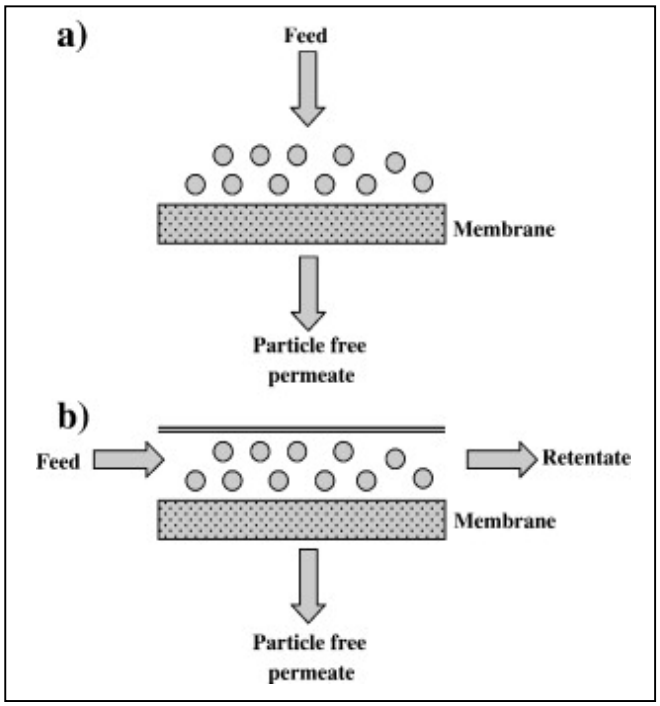

Figure 2.6: Comparison between: (a) dead-end, (b) cross-flow configuration

Microfiltration (MF) designates a membrane separation process similar to UF but with even larger membrane pore size allowing particles in the range of 0.2 to 2 micrometers to pass through. In MF, the pressure used is generally lower than that of UF process. MF is used for fermentation broth clarification and biomass clarification and recovery.

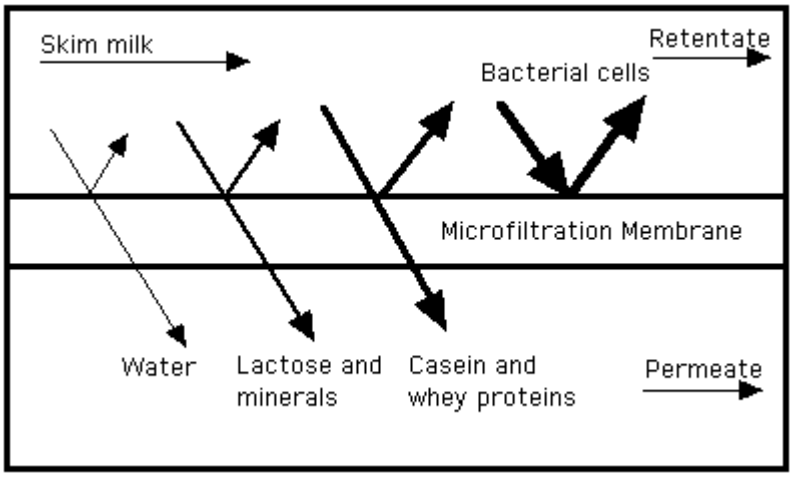

Figure 2.7: Microfiltration

2.2.2.3 Kvick Cassette

The Kvick cassettes (figure 2.8) intergrate a new internal design with materials that exhibit very low extractables. The membrane material is polyethersulfone (PES) and is resistant to commonly used chemicals.

Figure 2.8: Kvick lab and Kvick flow cassettes are available in a variety of membrane areas and molecular weight cutoffs to fit almost any application.

The membranes are precise and reproducibly selective, with sharp cutoffs. Kvick lab cassettes are available in molecular weight cutoffs of 10 kDa, 30 kDa, 50 kDa, and 100 kDa to fit a broad range of cross flow applications. Kvick lab cassettes are available with 0.01 m2 (0.1 ft2) or 0.12 m2 (1.25 ft2) of membrane surface area.

The innovative, patent pending anti-dead space design optimizes the efficiency of fluid flow and ensures that solutions reach all of the membrane surface area. This minimizes the risk of cross contamination and the potential of product loss in dead space surface area. Fluid paths are consistent across both Kvick Lab and Kvick Cassettes, allowing linear scale-up. An integrated gasket precludes the need to install separate gaskets between cassettes.

2.2.2.4 Driving Forces for Flow

During the course of filtration a number of phenomena occur at the membrane surface. The permeate flow through the membrane is driven by transmembrane pressure (ΔP_{TM}) or the average pressure difference between the feed and permeate sides of the membrane;

$$TMP = \frac{P_{in} + P_{out}}{2} + P_p \tag{2.1}$$

where P_{in} is the feed pressure (psi), P_{out} is the retentate pressure and P_p is the permeate pressure (Chollangi and Hossain., 2007).

Typical values of transmembrane pressures required for various types of membrane filtrations are shown in Table 2.4. The required pressure depends on the average pore size of the filtration membrane. Microfiltration membranes with relatively large pores required the least transmembrane pressure to achieve reasonable permeate fluxes. Ultrafiltration membranes that retain large molecules such as proteins but small solutes required significantly higher values of transmembrane pressure to achieve permeation compared with microfiltration membranes (Subramaniam, 2007).

Table 2.5: Typically used values of transmembrane pressures

Operation	Transmembrane pressure (bar)
Microfiltration	<1
Ultrafiltration	1-10
Reverse Osmosis	30-80

2.2.2.5 Permeate Flux

Permeate flux J is a measure of the volume flow rate of the permeate through the membrane per unit area of membrane; thus:

$$J = P/A \tag{2.2}$$

where P is the flow rate of the permeate and A is the area of the membrane. The permeate flux has the units of $m^3 m^{-2} s^{-1}$ (or $m s^{-1}$).

2.2.3 Conventional Way of Protein Isolation

Isolation is one of the techniques that we can use for protein separation. This technique is actually very easy to isolate proteins and requires very few steps compared to isolation of DNA or RNA. We need to be careful when isolating proteins as the tissue and proteins need to be kept cold to prevent them from degrading.

Milk contains a high proportion of proteins. The caseins have nutritive function whereas others have specific physiological functions in the mammary gland and in the neonate (Sánchez *et al.*, 1992a). The two iron-binding proteins found in the milk of many species are lactoferrin and transferring, which have been proposed to be involved in different biological functions. However, these proteins are not always present simultaneously (Hoshino *et al.*, 1996).

Milk is a complex bio-colloid which presents some unique problems for the protein isolation chemist, but the majority of the processing criteria for purifying recombinant proteins are the same as with any complex biological mixture. The casein in micelles and fat globules behave as separate phases; they prevent filtration of the milk and interfere with the usual separation methods (Tracy D Wilkins *et al.*, 1992).

CHAPTER 3

MATERIALS AND METHOD

3.1 Introduction

This chapter will discuss about processes to separated milk protein based on several parameters. Protein can be separated by using three types of separation technique. There are ultrafiltration (UF), microfiltration (MF), and protein isolation. Amount of protein attached was determined using Lowry method.

3.2 Research Procedure

3.2.1 Cross-flow filtration (CFF) system

The CFF system used in this study was a batch unit with partial recycle of retentate. Precautions were taken to stop foaming during filltration due the presence of entrapped air. A centrifugal pump (Reliance, Columbus, IN., 5.593 kW, 3510 rpm) was used as a feed pump and to control the flow and pressure. A co-concentric heat exchanger, in which skim milk circulated in the inner tube and cold water in the outer tube, was used to maintain constant temperatures. Pressure and retentate flow rates were adjusted by using two valves placed before the inlet and after the outlet. Pressures (inlet and outlet), retentate flow rate and inlet temperature were monitored and adjusted to remain closer to the selected values whenever necessary. Permeate weight was continuously taken and recorded against time to calculate permeate flux.

Each run was continued until the volume of retentate reached the desired concentration factor (CF). Permeate and retentate samples were taken at different CFs for analysis (Punidadas *et al*., 1998).

3.2.2 Lactoferrin Isolation

Lactoferrin is one of the iron-binding proteins are found in milk. For isolated the lactoferrin, milk was skimmed by centrifugation at 2500 ×g at 4 °C for 30 min. Skim milk was then diluted 1:1 with the dilution buffer (0.04 M NaH_2PO_4, 0.8 M NaCl, 0.04% (v/v) Tween 20, pH 7.4) and it was batch-wise incubated with SP-Sepharose at 4 °C overnight. Afterwards, the SP-Sepharose was washed with the washing buffer (0.02 M NaH_2PO_4, 0.4 M NaCl, 0.02% (v/v) Tween 20, pH 7.4) to elute the unbound proteins. The gel then packed into a column (5 × 30 cm or 3 × 30 cm, depending on the milk volume) and lactoferrin was eluted with the elution buffer (0.02 M NaH_2PO_4, 1 M NaCl, pH 7.4). The column was run at a flow rate of 3 mL/min (Celia Conesa *et al*., 2008).

3.3 CFF (Lab Procedure)

3.3.1 Milk Preparation

Milk was obtained from supermarket Type of milk that we used for this experiment is full cream milk (Dutch Lady). A centrifugation procedure was applied to separate cream from milk. Milk is centrifuge at 8000 rpm and 20°C for 30 minutes based on the step-wise centrifugation procedure of soymilk (Amir Malaki Nik *et al*., 2007). The advantages of this procedure are to prevent fouling at membrane cassette that we used during the experiment of crossflow filtration.

3.3.2 Equipment Set-Up and Membranes

The Kvick Lab Cross-Flow System with membranes of two different sizes (10 and 50 kDa) of generated polysulfone membranes with 0.11 m^2 were obtained from Amersham Biosciences. The solution will pumped through the system using a high pressure pump. The pressures were regulated using digital pressure gauges. The flow rate of retentate and permeate solutions will measure using measuring cylinder and stopwatch.

3.3.3 Experimental Procedures

The experiments were conducted in a closed loop system where the retentate from the membrane are not recycling back to the feed. There are five stages for running the experiment. The stages are initial water flushing stage, measuring water flux stage, sample filtration stage, final water flushing stage, cleaning and storage stage.

3.3.3.1 Initial Water Flushing

Before using a new cassette, the storage solution inside the Kvick Lab self-contained unit (SCU) should be removed. The reservoir is filled with deionized water for circulated the permeate and retentate. During the initial water flushing, the pump speed and retentate back pressure valve is adjusted to maintain a transmembrane pressure of 5 psi.

3.3.3.2 Measuring Water Flux

Measuring water flux involves measuring the flow of water through the SCU's membrane under controlled condition. The reservoir is filled with a volume of deionized water. The pump speed and retentate back pressure valve is adjusted based on the transmembrane pressure that we used. To remove entrained air, circulate the water 2 to 3 minutes at a crossflow rate of about 1200 L/min. From the measurement of water flux, we can know the effectiveness of the cleaning cycle.

3.3.3.3 Sample Filtration

The reservoir is filled with sample of milk. The solution is filtered by pumped it through the membrane unit. During this stage, the feed pressure and the retentate pressure were changed adequately to give transmembrane pressure of 10-30 psi. At each transmembrane pressure, the permeate flow and retentate was measured and the samples at both of side were collected every minutes for analysis.

3.3.3.4 Final Water Flushing

Final water flushing is similar to the initial water flushing stage. It is essential to ensure that there is no dissolved fouling material in the wash solution.

3.3.3.5 Cleaning and Storage

To clean a used Kvick Lab SCU, drain it and then rinse it with deionized water first. Then clean the Kvick Lab SCU with sodium hydroxide reagent solution. This solution were circulated and physically removed contaminates from the Kvick Lab SCU. After the circulating process, rinsing with water removes the cleaning solution from the Kvick Lab SCU. The storage solution recommended for this membrane cassette is 50 M sodium hydroxide and stored it for about three days and was used in all experiments.

3.4 Isolation of Protein (Lab Procedure)

Prepared the milk sample by centrifuge at 2000 rpm and 20°C for 10 minute to remove fat layer and used the solution for the test. The solution of milk sample is diluted with distilled water and kept in water bath at 30°C. Add 0.4 M acid hydrochloric until pH 4.6 is obtained. The filtration through cheese cloth required to collect the precipitated milk protein. The amount of wet and dry sample is measured until it attains a constant weight.

3.5 Analytical Procedure

3.5.1 Protein Assay (Lowry Method)

The amount of protein content after the separation process of milk protein was determined by using the method of Lowry's method. The principle of this method is based on the facts that the Folin-Ciocalteun reagent reacts with aromatic residues of protein and yields a blue color which is turn is read in a colorimeter. Blue color develops because the alkaline copper reacts with proteins.

3.5.2 Lowry's Method Procedure

Lowry solution was prepared by mix reagent A and reagent B in a 50:1 ratio and folin solution was prepared by dilution of Folin reagent to distilled water with ration 1:1. Standard curve then was prepared by preparing a series of dilution for BSA stock solution at concentration of 200, 500, 1000, 1500 and 2000 μg/ml. 1.0 ml of modified Lowry reagent was added into 0.2 ml of each concentration of BSA. The mixture was mixed well and leave at room temperature. After 10 minutes, 0.1 ml 1 N Folin-Ciocalteun reagent is added into the mixture and leave it for 30 minutes. After 30 minutes, the absorbance of the sample is measured at 750 nm against blank. Calibration curve of optical density (OD) versus concentration for each BSA concentration was plotted. The same procedure was repeated to determine protein concentration in sample by replacing the 0.2 ml of BSA solution with 0.2 ml of milk sample.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

There are many types of protein separation can be used for milk protein separation. However, the main concern will be the quantity of milk protein that will be separated and the efficiency of each method. The choosing of the membrane is most important to make sure that the operation process is successful. This chapter looks is discuss about the factor of the pore size of the membrane and its operation condition.

4.2 Quantitative Protein Estimation

Lowry gave a simple and less time-consuming colorimetric method for quantitative estimation of proteins. The principle of this method is based on the facts that the folin-ciocalteau reagent reacts with aromatic residues of protein and yield a blue color which in turn is read in a calorimeter. Blue color develops because the alkaline copper reacts with protein.

Below is the table of data from spectrophotometer and use for construction of standard curve of Lowry's method.

Table 4.1: Concentration of BSA versus Optical Density

BSA concentration (µg/ml)	Optical Density (1st)	Optical Density (2nd)	Optical Density (3rd)	Average Concentration
0	0.000	0.000	0.000	0.000
200	0.518	0.427	0.432	0.459
500	0.867	0.879	0.885	0.877
1000	1.445	1.486	1.513	1.481
1500	1.967	2.310	2.366	2.214
2000	3.000	3.000	3.000	3.000

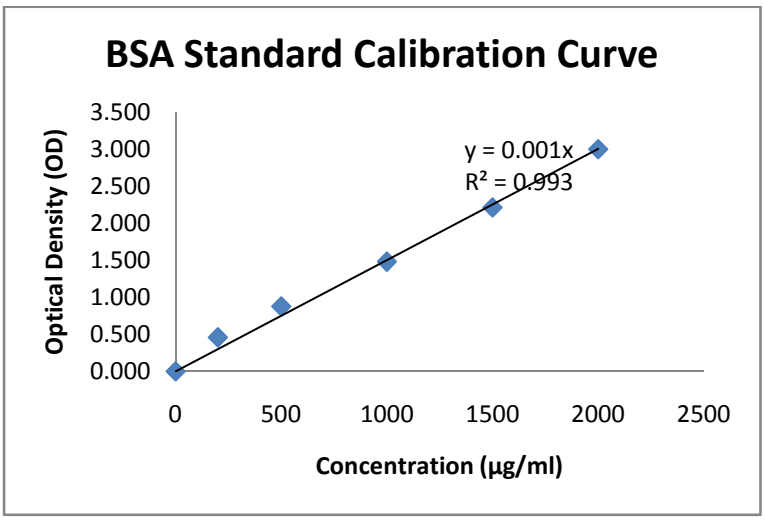

Figure 4.1: Concentration of BSA versus Optical Density

The calibration curve of bovine serum albumin (BSA) is used to determine the concentration of protein. From the figure 4.1, it shows that the optical density increased linearly to the concentration of BSA. BSA is a standard protein that used to determine the normally concentration of the other protein.

4.3 Crossflow Filtration

4.3.1 Water Flux

Measuring water flux involves measuring the flow of water through the membrane under controlled process conditions. Water flux was measured at the beginning and after the experiment. The water flux value obtain provided an indicator of the performance of membrane and effectiveness of cleaning cycles. Below is the table of data from the measurement of water flux for two types of membrane cassette. There are 10kDa and 50kDa molecular weight cutoff of membrane (MWCO).

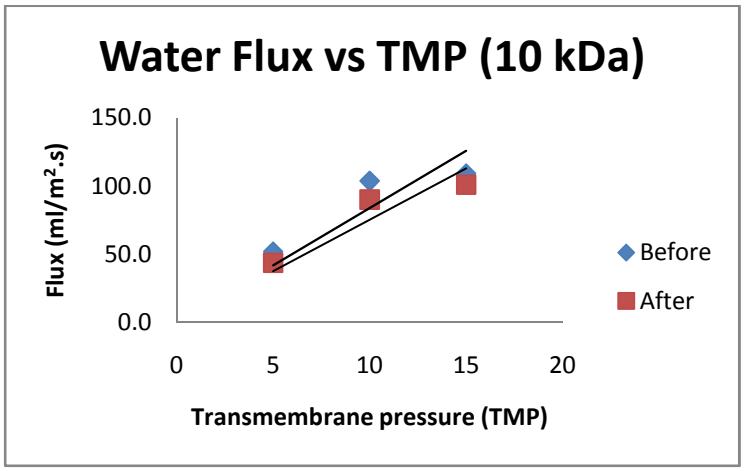

Figure 4.2: Plot of water flux versus transmembrane pressure for 10 kDa membrane

Figure 4.2 shows that the profile of water fluxes versus transmembrane pressure for 10 kDa MWCO membrane cassette. Water flux was measured at the beginning and the end of the experiment. Based on the graph, it can be observed that the water flux was increased linearly with the test range of 5 to 15 psi transmembrane pressure. The water flux before experiment is high than the water flux after the experiment. This is expected that some of the component from the sample of milk would have absorbed on to the membrane surface and cause such decrease, but the range of flux decreased is very small. So that, the performances of 10 kDa MWCO membrane cassette is still in the good condition.

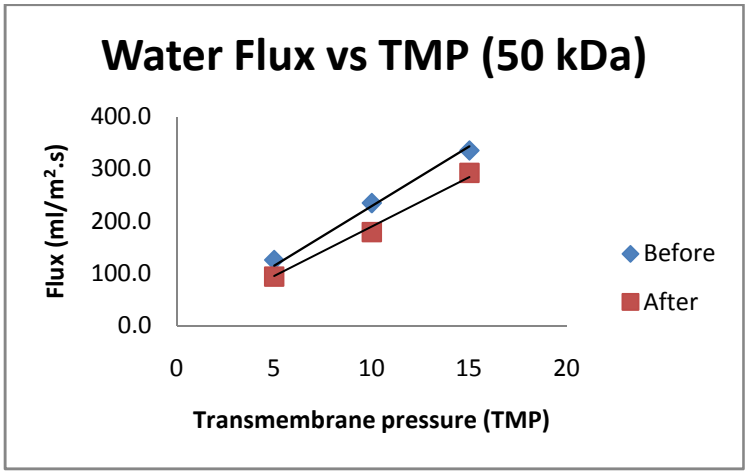

Figure 4.3: Plot of water flux versus transmembrane pressure for 50 kDa membrane

Based on the graph from figure 4.3, the water flux was increased by increasing the transmembrane pressure for 50 kDa MWCO membrane cassette. The water flux before the experiment is higher than the water flux after the experiment same condition with 10 kDa MWCO membrane cassette. Although the water flux before and after experiment was different, but the different was in a small range. So, the 50 kDa is also in a good condition. It shows that the performance of both membrane cassettes is good and can be used for the protein separation. From the water flux measurement, the cleaning cycles of the membrane cassette was more effectives based on the increasing of the water flux.

4.3.2 Crossflow filtration by molecular weight cutoffs of 10 kDa and 50 kDa

4.3.2.1 Effect of Membrane Size

Figure 4.4 shows the effect of transmembrane pressure with permeate flux at both of 10 kDa and 50 kDa membrane size. It can be observed that the permeate flux decline slightly with the operating times. For membrane of MWCO of 50 kDa, at the start of the operation, the flux were high, however at the times over 240 seconds, the permeate flux reach limiting flux.

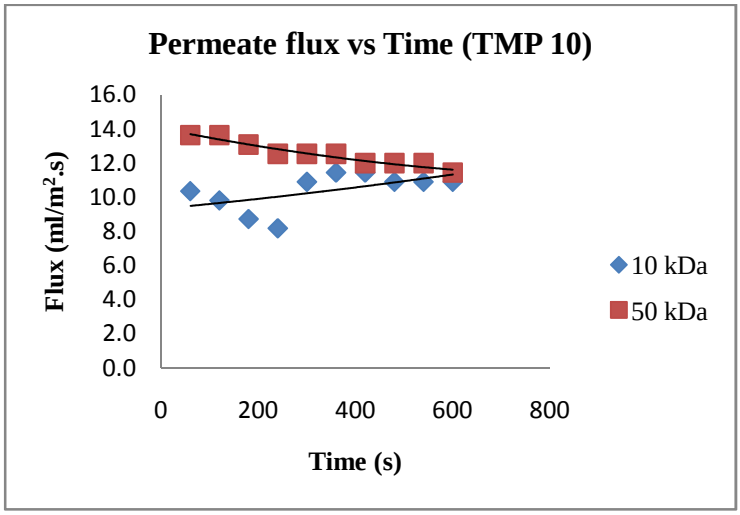

Figure 4.4: Permeate flux versus time at transmembrane pressure of 10 psi.

For membrane of MWCO of 10 kDa, at the start of the operation, the flux was high. At the first 240 seconds the flux was decreased by decreasing the time. However, the flux was increase at the time of 300 second. It is because some error occurred when adjusting the pump speed to maintain the transmembrane pressure. The permeate flux become steady at 500 second. The reduction of flux is because of the concentration of the solute and fouling.

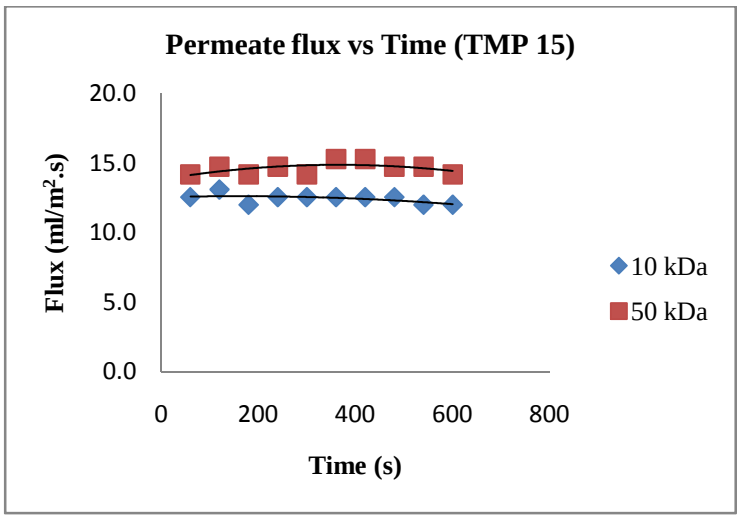

Figure 4.5: Permeate flux versus time at transmembrane pressure of 15 psi.

Figure 4.5 shows that the permeate flux of the MWCO of 50 kDa membrane was decreased by decreasing the time. The decreasing of flux is not consistent. It is different with MWCO of 10 kDa membrane where at the first 200 second, the

permeate flux was decreased and it reached limiting flux at 250 seconds. This is caused by concentration polarization. Concentration polarization is the increased in solute concentration that occurs at a membrane surface. The raised particulate concentration increases the resistance to solvent flow and thus reduces the flux.

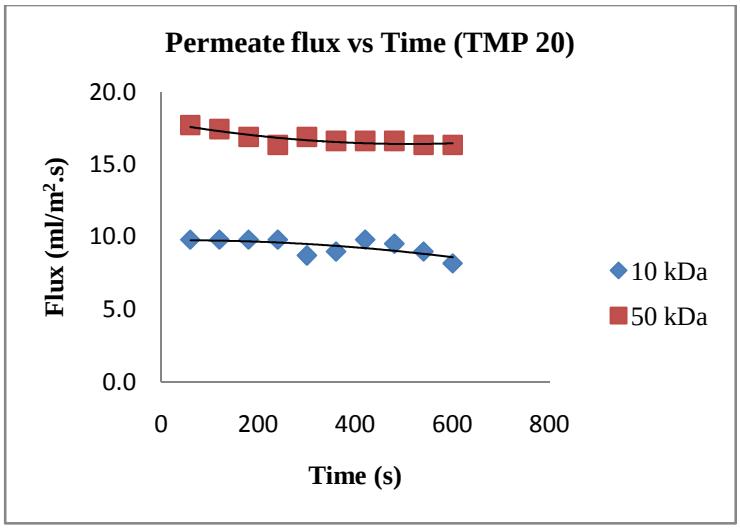

Figure 4.6: Permeate flux versus time at transmembrane pressure of 20 psi.

Figure 4.6 shows the effect of transmembrane pressure of 20 psi with permeate flux at both of 10 kDa and 50 kDa membrane size. Permeate flux of the MWCO of 50 kDa membrane was higher than MWCO of 10 kDa membrane. Permeate flux for both membrane cassettes were decrease by decreasing the time. For 50 kDa membrane, the flux become steady at 250 second but for the 10 kDa membrane, the flux was steady at the first process separation. After 400 second for the 10 kDa membrane, the flux was decreased. It shows that fouling is responsible to drob the performance of the membrane.

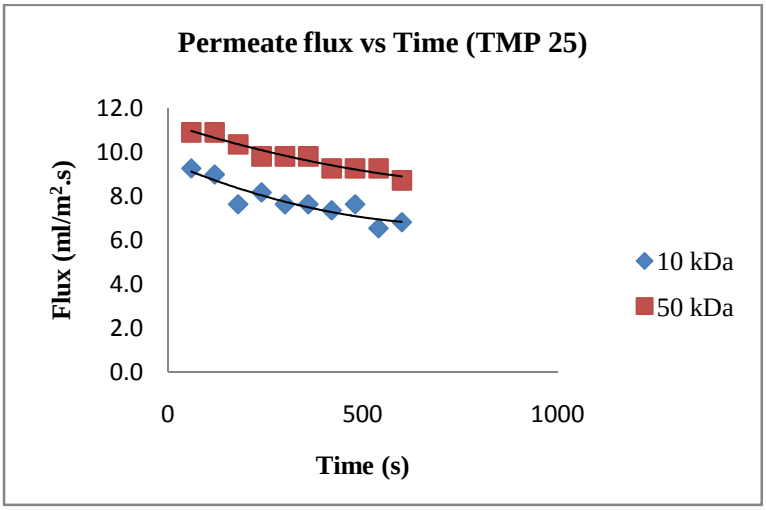

Figure 4.7: Permeate flux versus time at transmembrane pressure of 25 psi.

Based on the graph above, (Figure 4.7) it can be observed that the MWCO of 50 kDa membrane has given high flux rather than 10 kDa. The permeate flux were decrease by decreasing the time. It can be describe that generally, larger pore size membranes give slightly higher flux, provided that pore size is substantially less than the particle of solute size.

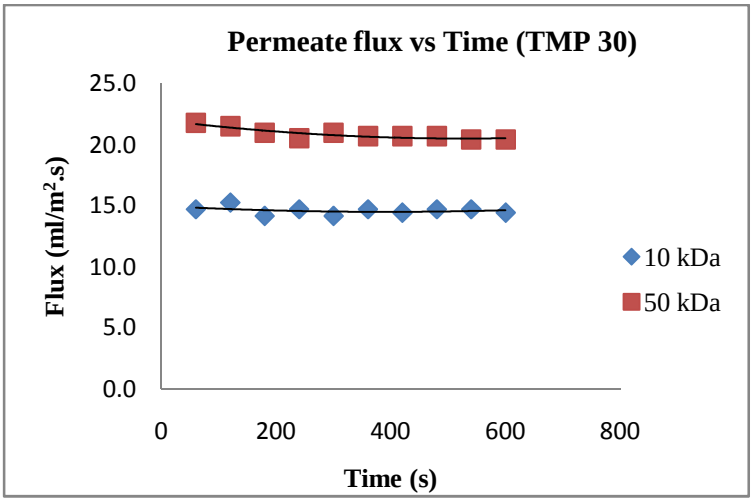

Figure 4.8: Permeate flux versus time at transmembrane pressure of 30 psi.

Figure 4.8 shows the effect of transmembrane pressure of 30 psi with the permeate flux for both of 50 and 10 kDa membrane. The graph describe that the reduction of the flux is slightly slow. This condition was occurred because the adsorption of the solutes was reached at the maximum value cause result of limiting

in the flux. There shows that the pore of the membrane was blockage where the particles become lodged at pore entrances.

4.3.2.2 Transmembrane pressure effect

In order to assess the effect of transmembrane pressure (an experimental mechanical parameter in milk UF) a series of experiments were recorded at different transmembrane pressure (TMP) in 10 – 30 psi range, at constant temperature (20°C). Apply equation (2.2), for permeate flux at different applied transmembrane pressure was obtained. Permeate flux profiles showed an expected decline in the whole process. Below is the figure of the relationship between permeate flux and time for every transmembrane pressure.

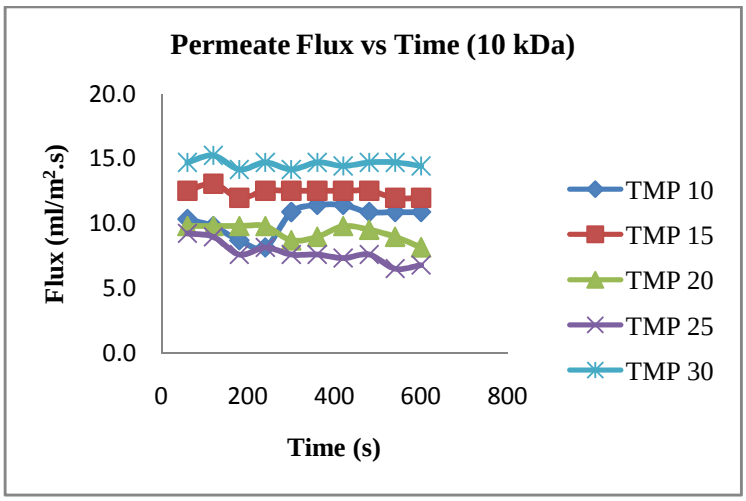

Figure 4.9: Permeate flux in milk as a function of time for 10 kDa MWCO membrane cassette

Based on the graph above (Figure 4.9), it can be observed that the permeate flux decreased with time at transmembrane pressure range of 10–30 psi. The graph shows that the high permeate flux is at transmembrane pressure of 30 psi and the low permeate flux is at transmembrane pressure of 25 psi. At the initial process, the permeate flux was increase by increasing the transmembrane pressure from 10 psi until 15 psi. After that, the permeate flux was decreased at transmembrane pressure

of 20 psi until 25 psi and increased back at transmembrane pressure of 30 psi. The initial fouling occurred at transmembrane pressure of 20 psi.

This is expected as the component from the milk sample would have absorbed on to the membrane surface and cause such decrease. The 10 kDa MWCO membrane was clogged and the permeate cannot flow through the membrane cassette. Although the permeate flux at transmembrane pressure of 30 psi is very high, but the best transmembrane pressure is 15 psi. It is because; at transmembrane pressures of 30 psi, content in permeate was mixed with the component from retentate.

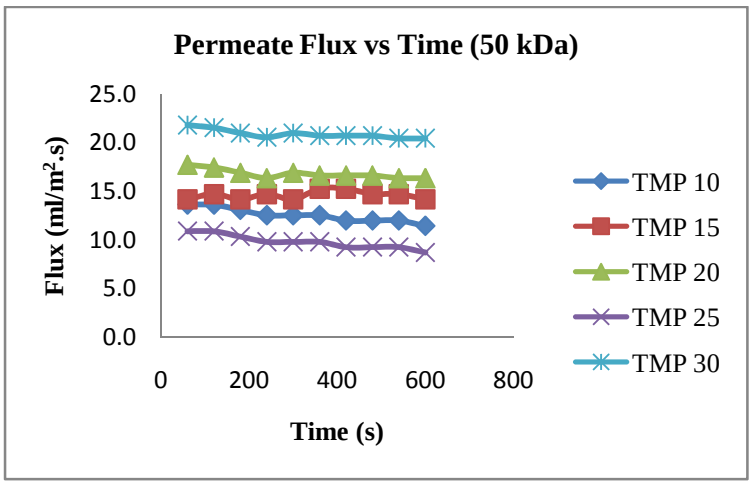

Figure 4.10: Permeate flux in milk as a function of time for 50 kDa MWCO membrane cassette

Based on the graph above (Figure 4.10), it can be observed that the permeate flux decreased with time at transmembrane pressure range of 10–30 psi. The graph shows that the high permeate flux is at transmembrane pressure of 30 psi and the low permeate flux is at transmembrane pressure of 25 psi same with 10 kDa MWCO membrane cassette. At the initial process, the permeate flux was increase by increasing the transmembrane pressure from 10 psi until 20 psi. After that, the permeate flux was decreased at transmembrane pressure of 25 psi and increased back at transmembrane pressure of 30 psi. The initial fouling occurred at transmembrane pressure of 25 psi.

This is expected that the fouling occurred because some component from milk that clogged at the membrane surface. A compressible filter cake is present on the surface of the membrane. The best transmembrane pressure for this membrane cassette was at 20 psi. The steady state has been achieved after initial fouling, that is the rate of adsorption of molecules on the membrane surface is equal to the rate of molecules removal from the surface due to the tangential flow.

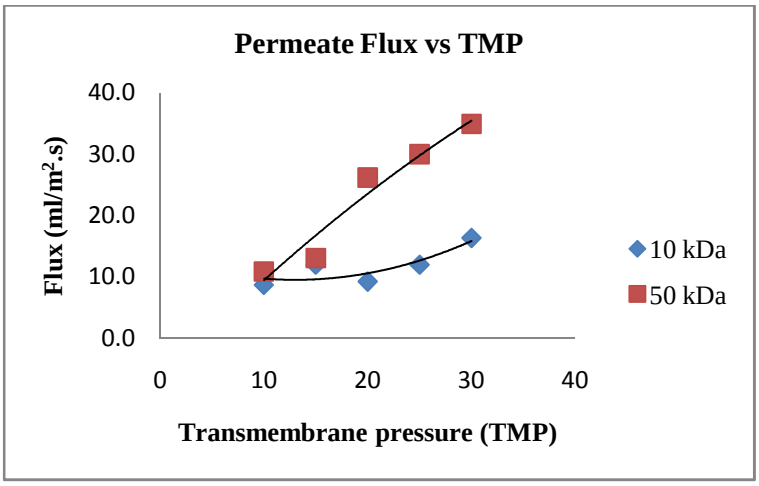

Figure 4.11: Permeate flux versus transmembrane pressure (psi), comparison of two types of membrane cassette

Figure 4.11 shows the effect of transmembrane pressure on permeate flux with two types of membrane cassette: (i) MWCO 10 kDA and (ii) MWCO 50 kDa. It can be observed from the results with both of membranes cassette that the permeate flux increased with increasing transmembrane pressure. This is expected as the increase in transmembrane pressure increases the driving force across the membrane, and give higher permeate flux. However, the flux for the molecular weight cutoffs of (MWCO) 50 kDa was greater than molecular weight cutoffs of (MWCO) 10 kDa at all transmembrane pressure.

By comparison of 10 kDa and 50 kDa MWCO membrane cassette, the best transmembrane pressures are 15 and 20 psi. The initial fouling occurred fast for 10 kDa compare with 50 kDa. This is occurred because of the pore size of both membranes cassette. Pore size of 10 kDa membrane is smaller than 50 kDa

membrane. So, the material that pass through the small membrane size easy to attached at the membrane surface compare with large membrane size.

4.3.2.3 Concentration effect

From the BSA standard calibration curve, the concentration of the milk sample can be determined. Graph of the milk sample concentration versus time can be plotted. The protein concentration also decreased with time (Figure 4.12), almost similar with the flux. This is the effect of fouling due to the protein present in the milk sample causing a decrease in the permeate flow and protein content in it.

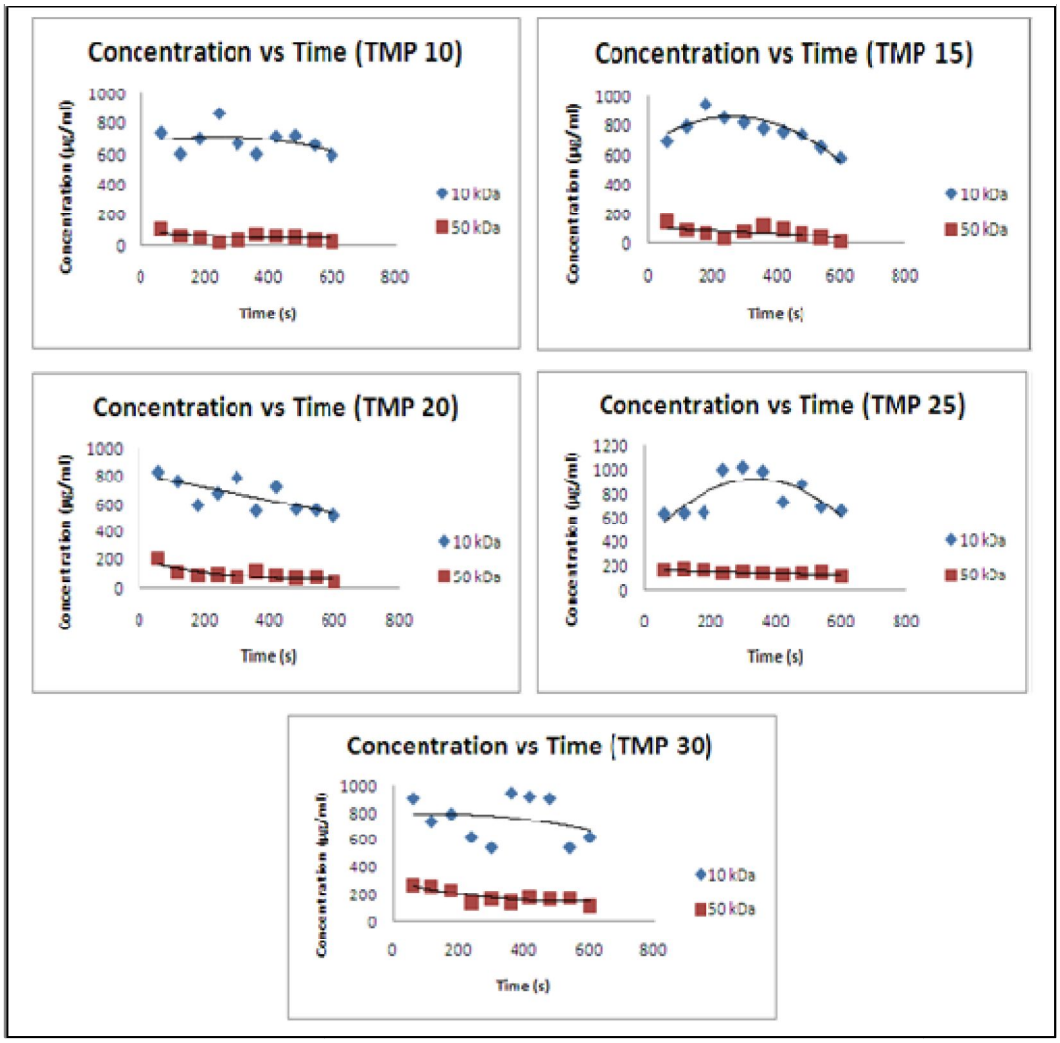

Figure 4.12: Concentration of milk as a function of time for transmembrane pressure of 10 – 30 psi for; (i) 10 kDa MWCO membrane and (ii) 50 kDa MWCO membrane

Based on the graph above, it can be observed that the concentration of milk sample was decreased by increasing the time. The value of decreased was in a small range for both of membrane cassette, along 10 minutes separation processes. By comparison of both membrane cassettes, the high concentration of milk sample achieved at 10 kDa MWCO membrane cassette. It is believed that the different concentration for both membrane cassettes occurred because of the membrane pore sizes. .

According to the graph above, the quantity of milk can be determined based on the concentration of the milk sample. By using equation (4.1) the mass of protein precipitated can be determined. The amount of protein content in milk sample can be determined for both of membrane cassette.

Mass (gram) = Concentration of protein (µg/ml) x permeate volume (ml) (4.1)

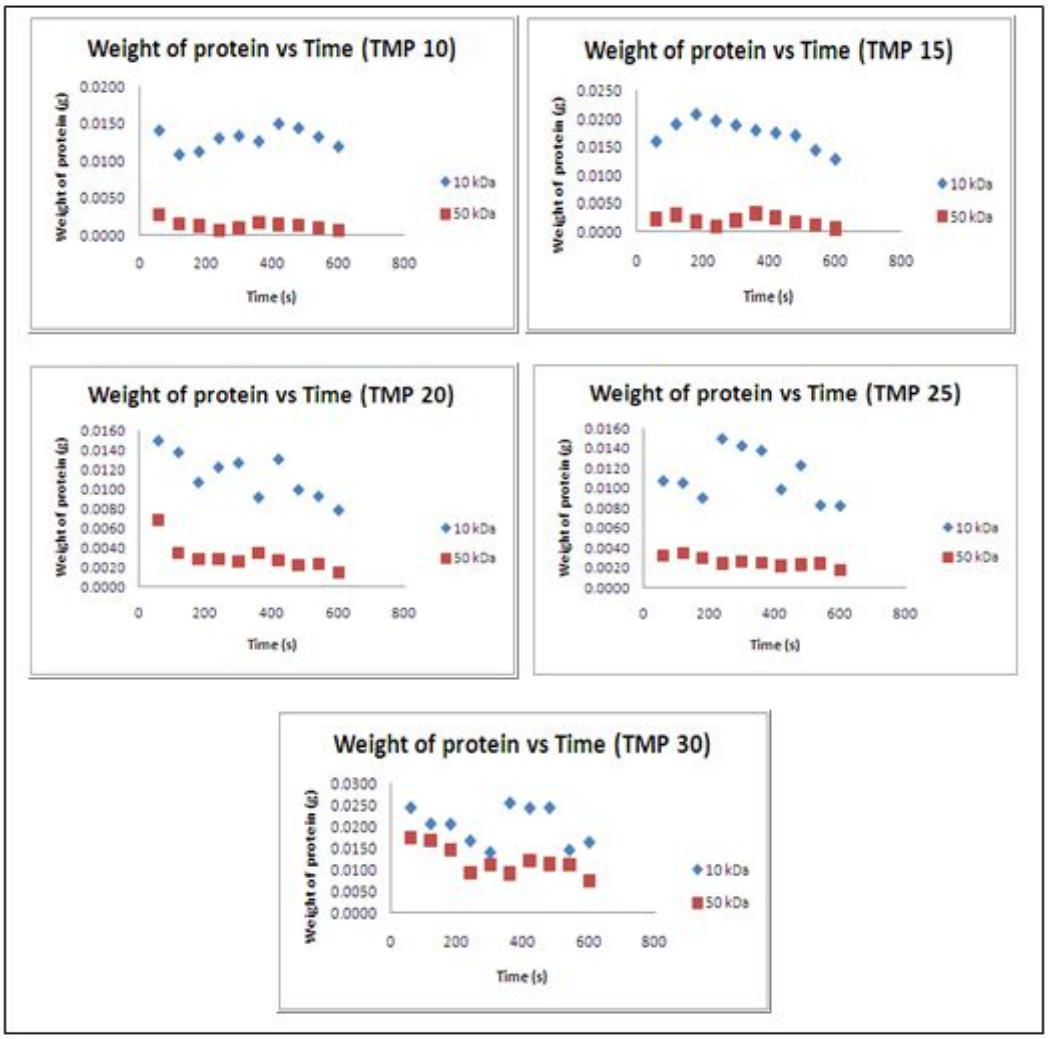

Figure 4.13: Weight of milk as a function of time for transmembrane pressure of 10 – 30 psi for; (i) 10 kDa MWCO membrane and (ii) 50 kDa MWCO membrane

Figure 4.13 shows the effect of membrane cassette on separation of milk protein for every transmembrane pressure. It can be observed from the results that the membrane cassette of MWCO value of 10 kDa is produce more protein compared with MWCO value of 50 kDa membrane. A MWCO value of 10 kDa means that the membrane can retain from a feed solution 90 % of the molecules having molecular weight of 10 kDa (Raja Ghosh, 2003).

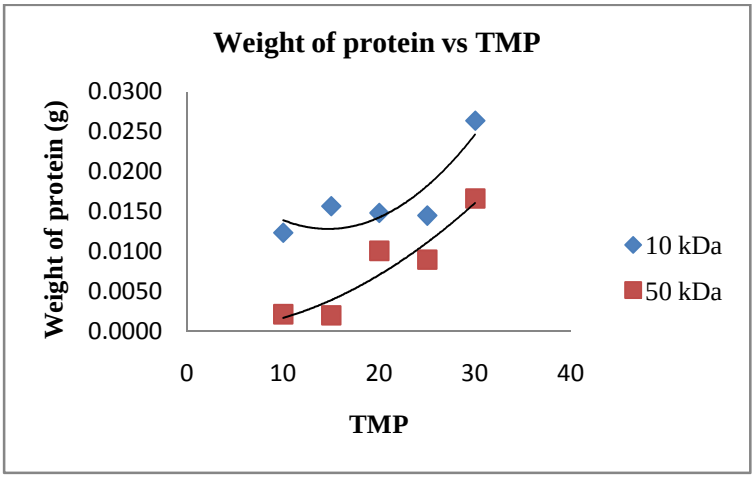

Figure 4.14: Weight of milk as a function of TMP for MWCO 10 kDa and MWCO 50 kDa

Figure 4.14 reveals that as the transmembrane pressure increased the weight of protein for both types of membrane cassette. However, the weight of protein for 10 kDa MWCO membrane cassette is greater than 50 kDa MWCO membrane cassette. At 10 kDa MWCO membrane, the high amount of protein achieved at transmembrane pressure of 15 psi. The quantity of milk protein then decreased at transmembrane pressure of 20 psi. Different with 50 kDa MWCO membrane, the high amount of protein achieved at transmembrane pressure of 20 psi and decreased at transmembrane pressure of 25 psi. Some error occurred at 10 psi for 50 kDa MWCO membrane. This is caused by adjusting of pump speed to maintain transmembrane pressure.

The graph shown that MWCO of 10 kDa membrane was ability to retain macromolecules more than MWCO of 50 kDa membrane. When a solution of macromolecules is filtered, the retained macromolecules accumulate near the membrane surface.

4.4 Protein Isolation

It is actually very easy to isolate proteins and required very few steps. Before filter the sample using the filter paper, the pH of the sample should be at 4.6. So, 0.4 M HCL was dropped continuously until the pH 4.6 was achieved. Table 4.2 shows the amount of 0.4 M HCL added into the milk sample for four trials.

Table 4.2: Amount of 0.4 M HCL added to the milk sample

	Initial		**Final**		
Sample	T (°C)	pH	T (°C)	pH	Amount 0.4 M HCl added (ml)
1st	20	7.18	20.8	4.57	3
2nd	20	7.17	21	4.62	3
3rd	20	7.14	20.9	4.61	3
4rd	20	7.17	20.8	4.59	3

After the pH 4.6 was obtained, the mixture of the sample was filtered through the filter paper. The precipitated casein curd was attached at the filter paper. By weight the wet and dry precipitated casein curd, the amount of protein can be determined.

Table 4.3: Amount of protein precipitated in for protein isolation method

			Precipitated casein curd	
Sample	Wet weight (g)	Dry weight (g)	Wet weight (g)	Dry weight (g)
1st	42.5663	31.3988	15.5720	4.4045
2nd	41.8325	30.1882	14.8382	3.1939
3rd	41.7673	29.9615	14.7730	2.9672
4rd	40.0061	30.0053	13.0118	3.0110
			14.5488	3.3942

The amount of protein precipitated can be determined by using equation 4.2. From the data in Table 4.3, it can be observed that, the average amount of protein precipitated by using the protein isolation method is around 3-4 grams in 50 ml sample.

Protein precipitated (g) = wet weight (g) – dry weight (g) – weight of filter paper (g) – weight of Petri dish (g)

(4.2)

4.5 Comparison on protein separation using three different techniques

According to those three methods of protein separation, the best method for protein separation can be determined. Table 4.4 shows that the comparison between the three techniques of protein separation based on the amount of protein, their handling and time to be taken during the experiment.

Table 4.4: Comparison of the three different technique of protein separation

	10 kDa MWCO membrane	50 kDa MWCO membrane	Conventional method
Mass	0.00078 g in 1 ml sample	0.00019 g in 1 ml sample	0.06788 g in 1 ml sample
Handling	Easy	Easy	Difficult
Time	Fast	Fast	Slow

From the table 4.4, it can be observed that the best method for protein separation is by using crossflow filtration technique with 10 kDa MWCO membrane cassette. This technique had more advantages compared with another technique. Although conventional method produce more protein, but the handling of this experiment was quietly difficult and take time. So, the best technique was separation of protein using 10 kDa MWCO membrane cassette because easy to handling the experiment and save time.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study reveals the potential use of membrane filtration as a mechanical separation process in fractionation of milk proteins. Among the experiments of three methods of protein separation, membrane of 10 kDa molecular weight cutoff off membrane (MWCO) was found to be good to separated protein from full cream milk. In this systems, increase in transmembrane pressure, increase significantly the permeate flux and the amount of protein retained in permeate solution.

The pore size of the membrane cassette can affect the process of protein separation. The pore size of 10 kDa MWCO membrane is smaller than the pore size of 50 kDa MWCO membrane. So that, the 10 kDa MWCO membrane can retained macromolecules more than 50 kDa MWCO membrane in permeate solution. When a solution of macromolecules is filtered, the retained macromolecules accumulate near the membrane surface.

According to the protein separation by using isolation method, the protein precipitated that was collected from the filter paper is more than amount that collected from the membrane separation. Although the amount of protein precipitated is large compare with the others method, but the process is not efficient because isolation method was taken a long time to separate protein.

5.2 Recommendation

This experiment was merely to study how to collect the high amount of milk protein by using the best technique of separation processes. From this research, the best technique is from separation using crossflow filtration with 10 kDa MWCO membrane cassette. The experiment is running during 10 minutes. The result of the experiment was not clear. As for future, it is advisable to operate the experiment at a long time. From that the effectiveness of the membrane can be determine.

Other than that, lowering the pH (using HCL) significantly reduced the fouling at long time, even at high temperatures. Lowering the temperature when filtering at natural pH, or lowering the pH when filtering at high temperature, therefore provides useful pathways to maintain flux during filtration.

REFERENCES

A. Hoshino, S. Hisayasu and T. Shimada. (1996). *Complete sequence analysis of rat transferrin and expression of transferrin but not lactoferrin in the digestive glands*. Comp. Biochem. Physiol. *B.* **113**, pp. 491–497.

Alberts, Bray, Johnson, Lewis, Raff, Roberts, Walter. (1998). *Protein Separation.* Garland Publishing, a member of the Taylor & Francis Group.

Amir Maliki Nik, Susan Tosh, Vaino Poysa, Lorna Woodrow, and Milena Corredig. (2007). *Physicochemical characterization of soymilk after step-wise centrifugation*. Food Research International, Volume 41, Issue 3, 2008, Pages 286-294.

Arunima Saxena, Bijay P. Tripathi, Mahendra Kumar and Vinod K. Shahi (2008). *Membrane Based Techniques for The Separation and Purification of Protein*. Advances in Colloid and Interface Science. Volume 145, Issues 1-2, 30 January 2009, Pages 1-22.

Celia Conesa, Lourdes Sánchez, Carmen Rota, María-Dolores Pérez, Miguel Calvo, Sebastien Farnaud, and Robert W. Evans (2008). *Isolation of lactoferrin from milk of different species: Calorimetric and antimicrobial studies*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, Volume 150, Issue 1, May 2008, Pages 131-139.

Chollangi and Md. M. Hossain. (2007) *Separation of Proteins and Lactose from Dairy Wastewater*. Chemical Engineering and Processing. 46 (5): 398-404

Georgia C. Lauritzen, Food and Nutrition Specialist. (1992). *Food and Nutrition.* Board, National Academy of Sciences, National Research Council. Page 3.

L. Sánchez, L. Luján, R. Oria, H. Castillo, D. Pérez, J.M. Ena and M. Calvo. (1992). *Synthesis of lactoferrin and transport of transferrin in the lactating mammary gland of sheep*. J. Dairy Sci. **75**, pp. 1257–1262.

P. Punidadas and S. S. H. Rizvi. (1998). *Separation of milk proteins into fractions rich in casein or whey proteins by cross-flow filtration*. Food Research International. Volume 31, Issue 4, May 1998, Pages 265-272.

Raja Ghosh. (2006). *Principle of Bioseparations Engineering*. Singapore. World Scientific Publishing Co. Pte. Ltd.

Subramaniam G. (2007). *Bioseparation and Bioprocessing Handbook*. WILEY-VCH Verlag BmbH & Co. KGaA Weinheim.

Tracy D Wilkins and William Velander. (1992). *Isolation of recombinant proteins from milk*. Department of Chemical Engineering Virginia Polytechnic Institute, Blacksburg, Virginia 24061.

APPENDIX

Condition:

Cassette = 10 kDa

Milk = Full Cream Milk (Dutch Lady)

Table A.1: Pump Speed for Every TMP

TMP (psi)	Pump Speed (rpm)
5	173
10	220
15	270
20	315
25	370
30	405

Table A.2: Volume and Flux of Permeate at TMP 10 for both of membrane

	TMP = 10 psi			
	10 kDa		50 kDa	
Time(s)	Permeate Volume (ml)	Flux ($ml/m^2.s$)	Permeate Volume (ml)	Flux ($ml/m^2.s$)
0	0.0	0.0	0	0.0
60	19.0	10.4	25	13.6
120	18.0	9.8	25	13.6
180	16.0	8.7	24	13.1
240	15.0	8.2	23	12.5
300	20.0	10.9	23	12.5
360	21.0	11.5	23	12.5
420	21.0	11.5	22	12.0
480	20.0	10.9	22	12.0
540	20.0	10.9	22	12.0
600	20.0	10.9	21	11.5

Table A.3: Volume and Flux of Permeate at TMP 15 for both of membrane

	TMP = 15 psi			
	10 kDa		50 kDa	
Time(s)	Permeate Volume (ml)	Flux ($ml/m^2.s$)	Permeate Volume (ml)	Flux ($ml/m^2.s$)
0	0	0.0	0	0.0
60	23	12.5	26	14.2
120	24	13.1	27	14.7
180	22	12.0	26	14.2
240	23	12.5	27	14.7
300	23	12.5	26	14.2
360	23	12.5	28	15.3
420	23	12.5	28	15.3
480	23	12.5	27	14.7
540	22	12.0	27	14.7
600	22	12.0	26	14.2

Table A.4: Volume and Flux of Permeate at TMP 20 for both of membrane

	TMP = 20 psi			
	10 kDa		50 kDa	
Time(s)	Permeate Volume (ml)	Flux ($ml/m^2.s$)	Permeate Volume (ml)	Flux ($ml/m^2.s$)
0	0.0	0.0	0.0	0.0
60	18.0	9.8	32.5	17.7
120	18.0	9.8	32.0	17.5
180	18.0	9.8	31.0	16.9
240	18.0	9.8	30.0	16.4
300	16.0	8.7	31.0	16.9
360	16.5	9.0	30.5	16.6
420	18.0	9.8	30.5	16.6
480	17.5	9.5	30.5	16.6
540	16.5	9.0	30.0	16.4
600	15.0	8.2	30.0	16.4

Table A.5: Volume and Flux of Permeate at TMP 25 for both of membrane

	TMP = 25 psi			
	10 kDa		50 kDa	
Time(s)	Permeate Volume (ml)	Flux ($ml/m^2.s$)	Permeate Volume (ml)	Flux ($ml/m^2.s$)
0	0	0.0	0	0.0
60	17	9.3	20	10.9
120	16.5	9.0	20	10.9
180	14	7.6	19	10.4
240	15	8.2	18	9.8
300	14	7.6	18	9.8
360	14	7.6	18	9.8
420	13.5	7.4	17	9.3
480	14	7.6	17	9.3
540	12	6.5	17	9.3
600	12.5	6.8	16	8.7

Table A.6: Volume and Flux of Permeate at TMP 30 for both of membrane

	TMP = 30 psi			
	10 kDa		50 kDa	
Time(s)	Permeate Volume (ml)	Flux ($ml/m^2.s$)	Permeate Volume (ml)	Flux ($ml/m^2.s$)
0	0	0.0	0.0	0.0
60	27	14.7	40.0	21.8
120	28	15.3	39.5	21.5
180	26	14.2	38.5	21.0
240	27	14.7	37.7	20.6
300	26	14.2	38.5	21.0
360	27	14.7	38.0	20.7
420	26.5	14.5	38.0	20.7
480	27	14.7	38.0	20.7
540	27	14.7	37.5	20.5
600	26.5	14.5	37.5	20.5

Table A.7: Optical Density, Concentration and Mass at TMP 10 for 10 kDa

	TMP = 10 psi				
	10 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0.0	0.000	0.0	0	0.0000
60	19.0	0.111	74.0	740	0.0141
120	18.0	0.090	60.0	600	0.0108
180	16.0	0.105	70.0	700	0.0112
240	15.0	0.130	86.7	867	0.0130
300	20.0	0.100	66.7	667	0.0133
360	21.0	0.090	60.0	600	0.0126
420	21.0	0.107	71.3	713	0.0150
480	20.0	0.108	72.0	720	0.0144
540	20.0	0.099	66.0	660	0.0132
600	20.0	0.089	59.3	593	0.0119

Table A.8: Optical Density, Concentration and Mass at TMP 10 for 50 kDa

	TMP = 10 psi				
	50 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0	0.000	0.0	0	0.0000
60	25	0.032	21.3	107	0.0027
120	25	0.018	12.0	60	0.0015
180	24	0.015	10.0	50	0.0012
240	23	0.008	5.3	27	0.0006
300	23	0.012	8.0	40	0.0009
360	23	0.022	14.7	73	0.0017
420	22	0.019	12.7	63	0.0014
480	22	0.017	11.3	57	0.0012
540	22	0.012	8.0	40	0.0009
600	21	0.009	6.0	30	0.0006

Table A.9: Optical Density, Concentration and Mass at TMP 15 for 10 kDa

	TMP = 15 psi				
	10 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0	0.000	0.0	0	0.0000
60	23	0.104	69.3	693	0.0159
120	24	0.119	79.3	793	0.0190
180	22	0.142	94.7	947	0.0208
240	23	0.128	85.3	853	0.0196
300	23	0.123	82.0	820	0.0189
360	23	0.117	78.0	780	0.0179
420	23	0.114	76.0	760	0.0175
480	23	0.111	74.0	740	0.0170
540	22	0.098	65.3	653	0.0144
600	22	0.087	58.0	580	0.0128

Table A.10: Optical Density, Concentration and Mass at TMP 15 for 50 kDa

	TMP = 15 psi				
	50 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0	0.000	0.0	0	0.0000
60	26	0.025	16.7	83	0.0022
120	27	0.032	21.3	107	0.0029
180	26	0.019	12.7	63	0.0016
240	27	0.01	6.7	33	0.0009
300	26	0.022	14.7	73	0.0019
360	28	0.034	22.7	113	0.0032
420	28	0.026	17.3	87	0.0024
480	27	0.018	12.0	60	0.0016
540	27	0.012	8.0	40	0.0011
600	26	0.005	3.3	17	0.0004

Table A.11: Optical Density, Concentration and Mass at TMP 20 for 10 kDa

	TMP = 20 psi				
	10 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0.0	0.000	0.0	0	0.0000
60	18.0	0.125	83.3	833	0.0150
120	18.0	0.115	76.7	767	0.0138
180	18.0	0.089	59.3	593	0.0107
240	18.0	0.102	68.0	680	0.0122
300	16.0	0.119	79.3	793	0.0127
360	16.5	0.083	55.3	553	0.0091
420	18.0	0.109	72.7	727	0.0131
480	17.5	0.085	56.7	567	0.0099
540	16.5	0.084	56.0	560	0.0092
600	15.0	0.078	52.0	520	0.0078

Table A.12: Optical Density, Concentration and Mass at TMP 20 for 50 kDa

	TMP = 20 psi				
	50 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0.0	0.000	0.0	0	0.0000
60	32.5	0.063	42.0	210	0.0068
120	32.0	0.032	21.3	107	0.0034
180	31.0	0.027	18.0	90	0.0028
240	30.0	0.028	18.7	93	0.0028
300	31.0	0.024	16.0	80	0.0025
360	30.5	0.034	22.7	113	0.0035
420	30.5	0.026	17.3	87	0.0026
480	30.5	0.021	14.0	70	0.0021
540	30.0	0.023	15.3	77	0.0023
600	30.0	0.014	9.3	47	0.0014

Table A.13: Optical Density, Concentration and Mass at TMP 25 for 10 kDa

	TMP = 25 psi				
	10 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0	0.000	0.0	0	0.0000
60	17	0.095	63.3	633	0.0108
120	16.5	0.096	64.0	640	0.0106
180	14	0.097	64.7	647	0.0091
240	15	0.150	100.0	1000	0.0150
300	14	0.153	102.0	1020	0.0143
360	14	0.148	98.7	987	0.0138
420	13.5	0.110	73.3	733	0.0099
480	14	0.132	88.0	880	0.0123
540	12	0.104	69.3	693	0.0083
600	12.5	0.099	66.0	660	0.0083

Table A.14: Optical Density, Concentration and Mass at TMP 25 for 50 kDa

	TMP = 25 psi				
	50 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0	0.000	0.0	0	0.0000
60	20	0.049	32.7	163	0.0033
120	20	0.052	34.7	173	0.0035
180	19	0.047	31.3	157	0.0030
240	18	0.041	27.3	137	0.0025
300	18	0.044	29.3	147	0.0026
360	18	0.042	28.0	140	0.0025
420	17	0.039	26.0	130	0.0022
480	17	0.041	27.3	137	0.0023
540	17	0.043	28.7	143	0.0024
600	16	0.034	22.7	113	0.0018

Table A.15: Optical Density, Concentration and Mass at TMP 30 for 10 kDa

	TMP = 30 psi				
	10 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0	0.000	0.0	0	0.0000
60	27	0.136	90.7	907	0.0245
120	28	0.111	74.0	740	0.0207
180	26	0.119	79.3	793	0.0206
240	27	0.093	62.0	620	0.0167
300	26	0.081	54.0	540	0.0140
360	27	0.142	94.7	947	0.0256
420	26.5	0.138	92.0	920	0.0244
480	27	0.136	90.7	907	0.0245
540	27	0.081	54.0	540	0.0146
600	26.5	0.093	62.0	620	0.0164

Table A.16: Optical Density, Concentration and Mass at TMP 30 for 50 kDa

	TMP = 30 psi				
	50 kDa				
Time(s)	Permeate Volume (ml)	Permeate (OD)	Concentration (µg/ml)	Concentration (x 10)	Mass (gram)
0	0.0	0.000	0.0	0	0.0000
60	40.0	0.078	52.0	260	0.0104
120	39.5	0.075	50.0	250	0.0099
180	38.5	0.067	44.7	223	0.0086
240	37.7	0.042	28.0	140	0.0053
300	38.5	0.051	34.0	170	0.0065
360	38.0	0.041	27.3	137	0.0052
420	38.0	0.054	36.0	180	0.0068
480	38.0	0.051	34.0	170	0.0065
540	37.5	0.052	34.7	173	0.0065
600	37.5	0.035	23.3	117	0.0044

Table A.17 (a): Volume and Flux of Permeate at all TMP (10 kDa)

Time (s)	TMP	Permeate Volume (ml)	Permeate (OD)	Permeate Conc. (µg/ml)	Permeate Conc. (x 10)	Flux ($ml/m^2.s$)
0	0		0.000	0	0	0.0
60	10	16	0.116	77	773	8.7
120	15	22	0.107	71	713	12.0
180	20	17	0.131	87	873	9.3
240	25	22	0.099	66	660	12.0
300	30	30	0.132	88	880	16.4

Table A.17 (b): Concentration and Mass of Permeate at all TMP (10 kDa)

Permeate Volume (ml)	Permeate (OD)	Permeate Conc. (µg/ml)	Permeate Conc. (x 10)	Mass (gram)
0	0.000	0	0	0.0000
16	0.116	77	773	0.0124
22	0.107	71	713	0.0157
17	0.131	87	873	0.0148
22	0.099	66	660	0.0145
30	0.132	88	880	0.0264
107				0.0838

Table A.18 (a): Volume and Flux of Permeate at all TMP (50 kDa)

Time (s)	TMP	Permeate Volume (ml)	Permeate (OD)	Permeate Conc. (µg/ml)	Permeate Conc. (x 5)	Flux ($ml/m^2.s$)
0	0	0	0.000	0	0	0.0
60	10	20	0.032	21	107	10.9
120	15	24	0.025	17	83	13.1
180	20	48	0.063	42	210	26.2
240	25	55	0.049	33	163	30.0
300	30	64	0.078	52	260	34.9

Table A.18 (b): Concentration and Mass of Permeate at all TMP (50 kDa)

Permeate Volume (ml)	Permeate (OD)	Permeate Conc. (µg/ml)	Permeate Conc. (x 5)	Mass (gram)
0	0.000	0	0	0.0000
20	0.032	21	107	0.0021
24	0.025	17	83	0.0020
48	0.063	42	210	0.0101
55	0.049	33	163	0.0090
64	0.078	52	260	0.0166
211				0.0398