EFFECTS OF TRANSMEMBRANE PRESSURE (TMP) ON THE PERFORMANCE OF ULTRAFILTRATION (UF) MEMBRANE OF SOY PROTEIN

AHMAD KAMAL BIN HASSAN

UNIVERSITI MALAYSIA PAHANG

UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS

JUDUL : **EFFECTS OF TRANSMEMBRANE PRESSURE (TMP) ON THE PERFORMANCE OF ULTRAFILTRATION (UF) MEMBRANE OF SOY PROTEIN**

SESI PENGAJIAN: **2008/2009**

Saya **AHMAD KAMAL BIN HASSAN**
(HURUF BESAR)

mengaku membenarkan tesis Projek Sarjana Muda (PSM) ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut:

1. Hakmilik kertas projek adalah di bawah nama penulis melainkan penulisan sebagai projek bersama dan dibiayai oleh UMP, hakmiliknya adalah kepunyaan UMP.
2. Naskah salinan di dalam bentuk kertas atau mikro hanya boleh dibuat dengan kebenaran bertulis daripada penulis.
3. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian mereka.
4. Kertas projek hanya boleh diterbitkan dengan kebenaran penulis. Bayaran royalti adalah mengikut kadar yang dipersetujui kelak.
5. *Saya membenarkan/tidak membenarkan Perpustakaan membuat salinan kertas projek ini sebagai bahan pertukaran di antara institusi pengajian tinggi.
6. **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)

Alamat Tetap: No 29, Jalan 2A/5,
Taman BBS Fasa 2A,
68100 Batu Caves,
Selangor Darul Ehsan

Tarikh: 30 April 2009

_____ (TANDATANGAN PENYELIA)

SUREENA BINTI ABDULLAH
Nama Penyelia

Tarikh: 30 April 2009

CATATAN:
* Potong yang tidak berkenaan.
** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan 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 disertai bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan 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 : Sureena binti Abdullah

Date : .....

EFFECTS OF TRANSMEMBRANE PRESSURE (TMP) ON THE PERFORMANCE OF ULTRAFILTRATION (UF) MEMBRANE OF SOY PROTEIN

AHMAD KAMAL BIN HASSAN

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

I declare that this thesis entitled “Effects of Transmembrane Pressure (TMP) on the Performance of Ultrafiltration (UF) Membrane of Soy Protein” 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 : Ahmad Kamal bin Hassan

Date : 30 April 2009

To my family members,
my friends – reality or virtual,
my fellow colleagues,
and all faculty members

And for those who keep whispering…
"You can do it!!!"

ACKNOWLEDGEMENT

I would like to forward my sincere appreciation to my research project supervisor, Miss Sureena Abdullah for her countless guidance and support. For my academic advisor, Mr Rozaimi Abu Samah, your concern and faith in me during my studies is deeply appreciated.

Special thanks to my beloved campus, University Malaysia Pahang, especially to Faculty of Chemical & Natural Resources Engineering, for facilities provided during the execution of this research project. To all supporting staffs of the faculty and laboratory assistants, thank you.

My appreciation also goes to all my fellow colleagues and friends, who have been here through all these years. Thanks for all point of views shared, and helping hands offered in this research project. Our moments and experiences will always be something sentimental to be remembered.

Last but not least, to my family members. Thank you.

ABSTRACT

The use of membrane separations using ultrafiltration (UF) in the fractionation of soy protein from soy milk has generated considerable interest, recently. Compared to traditional methods, UF gives higher yield and superior functional properties of the soy protein, and also allows the recovery of active soybean trypsin inhibitor (STI) for medical purposes. The performance of the UF membrane; in term of permeate flux, concentration of protein transmitted and retention ratio, by manipulating transmembrane pressure (TMP) to fractionate soy protein from soy milk is studied. Commercial soy milk samples have been centrifuged and introduced to the UF system at various TMP value. Data from the permeate flux, concentration of protein transmitted and retention ratio have been manipulated to get idea on the performance of the UF membrane. Based on the results, the range of effective TMP to fractionate soy protein from soy milk is between 15 to 20 psi. In this TMP range, the permeate flux is between 25 and 30 LMH, the concentration of protein transmitted is between 1.38 and 2.85 mg/mL and the retention ratio is 90 percent.

ABSTRAK

Proses pemisahan membran menggunakan ultraturasan (UF) dalam memisahkan protein soya dari susu soya semakin mendapat perhatian lewat kebelakangan ini. Berbanding dengan cara tradisional, UF memberikan hasil yang banyak dan ciri berfungsi protein soya yang tinggi, di samping membenarkan perencat tripsin kacang soya (STI) untuk dikumpulkan. Prestasi membran UF dengan memanipulasikan tekanan antara membran (TMP) untuk memisahkan protein soya dari susu soya melalui fluks; kepekatan protein menembusi membran, dan nisbah penolakan membran, dikaji. Susu soya komersil terempar dilalukan pada sistem UF pada beberapa nilai TMP yang ditetapkan. Maklumat dari fluks; kepekatan protein menembusi membran, dan nisbah penolakan membran telah dimanipulasikan untuk mendapatkan gambaran mengenai prestasi membran UF. Berdasarkan keputusan, julat efektif TMP adalah antara 15 dan 20 psi. Dalam julat tersebut, nilai fluks adalah antara 25 dan 30 LMH, kepekatan protein menembusi membran adalah antara 1.38 dan 2.85 mg/mL dan nisbah penolakan membran adalah 90 peratus.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSA	- Bovine serum albumin
DI water	- Deionized water
kDa	- kiloDalton
M	- Molar
MWCO	- Molecular Weight Cut-Off
NaOH	- Sodium Hydroxide
OD	- Optical Density
PES	- Polyethersulfone
S	- Sieving coefficient
STI	- Soybean Trypsin Inhibitor
TMP	- Transmembrane pressure
UF	- Ultrafiltration

LIST OF SYMBOLS

%	- Percent
°C	- Degree Celcius
cm / s	- Centimeter per second
cm / s. kPa	- Centimeter per second kiloPascal
ft^2	- Feet squared
g	- Gram
g / mL.	- Gram per milliliter
kPa	- KiloPascal
L	- Liter
lb / in	- Pound per inch
LMH	- Liter per meter squared per hour
m^2	- Meter squared
m^3	- Meter cubic
mg / L	- Milligram per liter
mL	- Milliliter
m / s	- Meter per second
NaOH	- Sodium hydroxide
N m	- Newton meter
nm	- Nanometer
R_{et}	- Retention Ratio

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Soy milk is a soy product which is rich in protein and carbohydrates. It is extracted from ground soybeans to form a colloidal solution (Zhang *et al.,* 2005; Guo *et al.*, 1997). The major health benefit of soy products is their use as a dairy substitute in lactose intolerant patients. The enrichment of the protein fraction from soy products yields a high value protein concentrate with enormous economical potential in the health food industry. (Akoum *et al*., 2006).

The market for soybean protein products received a substantial boost in 1999 when the United States of America (USA) government allowed a health claim for food products containing at least 6.25 g of soy protein per serving can reduce the risk of heart disease. To meet the demand, a new generation of functional soy protein ingredients had to be created using innovative technology (Kumar *et al.,* 2004).

The use of membrane separations using ultrafiltration (UF) in the fractionation of soy protein from soy milk has generated considerable interest, recently. Compared to traditional methods, UF gives higher yield and superior functional properties of the soy product, in addition to the benefits of the non-thermal and non-chemical nature of the UF

process (Kumar *et al.,* 2004; Cheryan, 1998). Moreover, the recovery of active soybean trypsin inhibitor (STI) for medical purposes by UF has added significant economic exploitation of the soybean.

In optimizing the UF membrane process of soy protein from soy milk, membrane fouling is one of the most important factors that will affect the membrane performance (Furukawa *et al.,* 2008). Fouling or accumulation of materials on the membrane can be observed when the permeate flux in an UF process does not increased linearly with transmembrane pressure (TMP) beyond a certain point. The pressure range in which the permeate flux increases with increase in TMP is referred to as the 'pressure dependant region', a region where the membrane is at its optimum performance (Ghosh, 2003).

Therefore in this study, a range value of TMP was tested in order to identify the value of TMP that give a better performance to the UF membrane in fractionation of soy protein from soy milk.

1.2 Problem Statements

Conventional method of protein fractionation like chromatography and electrophoresis faced several problems in term of scale up and the expensive equipments (Ghosh, 2003). The interest in usage of UF process in protein fractionation has developed from past 2 decades, but this UF process is strongly influenced by operating parameters like TMP. The optimization of the process seems to be the only way to make the process perfect.

There are limited published papers discussed about the optimized condition in UF process under the effects of TMP, but none used soy milk as raw material. The only

research that has been done by manipulating TMP to investigate the performance of membrane is only to the model beer provided by Thomassen *et al.* 2005.

Thus this research is important to know the range of TMP that can be used to allow the membrane to perform at its best, in order to fractionate soy protein from soy milk.

1.3 Objective

The main objective of this research is to investigate the performance of the ultrafiltration (UF) membrane; in term of permeate flux, concentration of protein transmitted and retention ratio, by manipulating transmembrane pressure (TMP) to fractionate soy protein from soy milk.

1.4 Scope of Study

The soy milk is gone through the UF process at various TMP values ranging from 5 to 25 psi. Other parameters like membrane pose size, pH and feed temperature were held constant. The permeate flux and retention ratio at any given TMP value were calculated, along with the measurement of protein concentration transmitted and retained on the membrane. The results from parameters being tested remarked the best performance a membrane can achieve at any given TMP value.

CHAPTER 2

LITERATURE REVIEW

2.1 Protein Bioseparation

The phenomenal development of the modern biotechnology has made protein bioseparation more important at present moment than any other time before. Protein bioseparation refers to the recovery, isolation, purification, and polishing of protein products (Ghosh, 2003). The growing industry, demands more and more protein products in absolute purify form.

However, there are some characteristics of protein products that should be understand before the purification of protein can be done:

a. These products present at very low concentrations in their respective biological feed streams
b. These products present, along with large number of impurities that have chemical and physical properties similar to those of target product. Hence, bioseparation has to be selective in nature
c. The quality requirements for these products are frequently demanding.
d. These products are thermolabile, and hence many bioseparation techniques are usually carried out at sub-ambient temperature
e. These products are sensitive to operating conditions (such as pH and salt concentrations) and also to chemical substances (such as surfactants and

solvents). The biological products are susceptible to denature and other forms of degradation in extreme conditions (Ghosh, 2006).

Thus, it is important for protein bioseparation to combine high productivity with high selectivity of separation. Protein bioseparation also must be feasible at mild operating conditions.

2.2 Economic Aspects of Protein Bioseparation

It is widely recognized that protein bioseparation is technically and economically challenging. The successful commercialization of protein-based product is depended on protein bioseparation, as it often regarded as the critical limiting factor, which usually is a substantial fraction of the total cost of production for most products of biological origin. Table 2.1 shows the bioseparation cost as approximate proportion of cost production for certain protein based products.

Table 2.1: Cost of protein bioseparation (Ghosh, 2003)

Products	**Approximate relative price**	**Bioseparation cost as percent of total cost of production**
Food/additives	1	10-30
Nutraceuticals	2-10	30-50
Industrial enzymes	5-10	30-50
Diagnostic enzymes	50-100	50-70
Therapeutic enzymes	50-500	60-80

As clearly indicated by these figures, bioseparation cost is the major cost of total production cost. Thus, it is very important to develop cost-effective isolation and purification processes.

2.3 The Recovery, Isolation, Purification and Polishing Scheme

A Recovery, Isolation, Purification and Polishing, (RIPP) scheme is commonly used in bioseparation (Ghosh, 2006). Table 2.2 lists the categories of RIPP scheme with some of the most commonly used protein bioseparation techniques.

The strategy of this scheme involves use of low resolution techniques, for example precipitation, filtration, centrifugation, and crystallization first for recovery and isolation, followed by high-resolution techniques, for example affinity separation, chromatography, and electrophoresis for purification and polishing. The high-throughput, low-resolution techniques are first used to significantly reduce the volume and overall concentration of the material being processed. The partially purified products are then further processed by high-resolution low-throughput techniques to obtain pure and polished finished products.

However, this scheme also has its disadvantages which include high capital cost, high operational cost and also lower recovery of products. Development of membrane separation processes and any other new types of separation creates potential to avoid this conventional RIPP scheme.

Table 2.2: Protein bioseparation techniques (Ghosh, 2003)

High-productivity, low-resolution
Cell disruption
Precipitation
Centrifugation
Liquid-liquid extraction
Microfiltration
Ultrafiltration (UF)
Supercritical fluid extraction
High-resolution, low-productivity
Ultracentrifugation
Packed bed chromatography
Affinity separation
Electropheresis
Supercritical fluid chromatography
High-resolution, high-productivity
Fluidised bed chromatography
Ultrafiltration (UF)
Monolith column chromatography

Membrane processes give high throughput and can be fine-tuned or optimized to give very high selectivity. The use of these new techniques can significantly cut down the number of steps needed for bioseparation (Ghosh, 2006). Note that Ultrafiltration (UF) is listed in two categories since the resolution in an UF process depends very much on how it is operated.

2.4 Soybean and Soy Milk

Soybean (*Glycine max*) is a nutritional plant which being consumed world wide, especially in the Asia region. It is believed to contain high concentration of proteins (40–50 percent), lipids (20–30 percent) and carbohydrates (26–30 percent), with daily average consumption is 20 to 80 g among Asian (Hernández-Ledesma *et al.,* 2005)

Ground soybeans can be soaked and grinded with water to produce soy milk. As the popular beverage among Asian population, soy milk which is a turbid and colloidal solution, contains almost all of its components of the soy seeds like protein, lipid, and saccharides (Zhang *et al.,* 2005; Guo *et al.*, 1997). The milk is regarded as being nutritious and cholesterol-free health foods. It is an excellent economical dairy substitute in lactose intolerant patients. In addition, soy milk and soy related products are also used extensively in infant formulas (Akoum *et al*., 2006). Table 2.3 indicates the nutrition composition of soy milk.

Table 2.3: Nutrition composition of soy milk (Dunne, 1975)

	Soymilk		Soymilk
Measure	1 C	Total lipid, g	4.7
Weight, g	245	Total saturated, g	0.5
Calories	81	Total unsaturated, g	2.04
Protein, g	6.7	Total monosaturated, g	0.8
Carbohydrate, g	4.4	Cholesterol, mg	0
Fiber, g	3.2		
Vitamin A, IU	78	Tryptophan, g	0.11
Vitamin B1, mg	0.4	Threonine, g	0.28
Vitamin B2, mg	0.17	Isoleucine, g	0.35
Vitamin B6, mg	0.1	Leucine, g	0.6
Vitamin B12, mg	0		
Niacin, mg	0.36	Lycine, g	0.44
Pantothenic acid, mg	0.12	Methionine, g	0.1
Folic acid, mg	3.7	Cystine, g	0.1
Vitamin C, mg	0	Phenylalanine, g	0.37
Vitamin E, IU	0.04	Tyrosine, g	0.27
Calcium, mg	9.8	Valine, g	0.345
Copper, mg	0.3	Arginine, g	0.5
Iron, mg	1.4	Histidine, g	0.17
Magnesium, mg	47	Alanine, g	0.3
Manganese, mg	0.42	Aspartic acid, g	0.84
Phosphorus, mg	120	Glutamic acid, g	1.35
Potassium, mg	346	Glycine, g	0.3
Selenium, mg	3.2	Proline, g	0.4
Sodium, mg	29	Serine, g	0.35
Zinc, mg	0.56		

The effects of soybean products on health have gained lot of interests in recent decades. On 26 October 1999, The United States Food and Drug Administration (FDA)

authorized the Soy Protein Health Claim stating that 6.25 g of soy protein a day may reduce the risk of heart disease. Due to this health claim, the market is very responsive, that later allow the soybean foods continue to penetrate rapidly into western cultures and diets (Zhang *et al.,* 2005; Fukushima, 2001; Hermansson, 1978). Some other studies also stated that its consumption may alleviate menopausal symptoms (Hernández-Ledesma *et al.,* 2005; Messina, 2000), and reduce the risk of osteoporosis (Hernández-Ledesma *et al.,* 2005; Shetty *et al.,* 2004; Barnes *et al.,* 1991).

A study by Fournier *et al.,* 1998 also demonstrated an inverse association between diets containing high amounts of soybean products and low cancer incidence and mortality rates, particularly breast, colon and prostate cancer. Although the specific components that are responsible for this chemopreventive activity remain to be identified, isoflavones isolated from soybeans have been extensively studied (Hernández-Ledesma *et al.,* 2005; Shetty *et al.,* 2004). However, the capacity of soybean trypsin inhibitors (STI) for preventing cancer and other age-related disorders is recently receiving more attention (Hernández-Ledesma *et al.,* 2005; Omoni *et al.,* 2005).

2.5 Soybean Trypsin Inhibitors (STI)

Soybean trypsin inhibitor (STI) is the most prominent antinutritional factors that present in raw soybean, which can cause serious problems in processing of soy products (Akoum *et al.,* 2006). The two main STI in soybean are the Kunitz and Bowman–Birk inhibitors. The Bowman–Birk inhibitors have molecular weights of around 8 kiloDalton (kDa) (Malaki *et al.,* 2008; Sessa *et al.,* 2001) and studies on Kunitz inhibitors founded that they have a molecular weight of 20 kDa (Malaki *et al.,* 2008; Kim *et al.,* 1985).

STI, along with hemagglutinins, phytoestrogens, allergens and the raffinose and stachyose oligosaccharides, can pose serious health risks if not removed or de-activated during the processing of raw soybeans (Akoum *et al.,* 2006; Salunkhe, 1991; Wolf,

1983). It has been estimated that 40 percent of the growth inhibition caused by feeding a diet of raw soy to rats, is attributable to STI activity (Akoum *et al.,* 2006; Salunkhe, 1991; Kakade *et al.,* 1973).

Soybean processing preparations usually involved heat to ensure food safety and extend the shelf life of the product. This applied heat will inactivate the STI activity. However, this inhibition is reversible (Akoum *et al.,* 2006; Roychauduri *et al.,* 2003), and it is therefore extremely important to monitor STI activity not only during processing, but also as a vital step in quality control.

Although STI is an undesirable ingredient of soybean preparations, purified STI is used for a number of medical purposes (Akoum *et al.,* 2006; Huang *et al.,* 2004; Kobayashi *et al.,* 2004), for inhibition of serine proteases in biochemical extractions and reactions, and also for a variety of specialist uses.

As, soy protein mainly composed of glycinin (11S) and ß-conglycinin (7S), with molecular weight of 320-380 kDa and 180 kDa, respectively (Malaki *et al.,* 2008), the recovery of active STI from soybean preparations by UF could therefore add significantly to the economic exploitation of soybean and this challenge is currently receiving considerable attention.

The importance of soy milk and soy related products can therefore be summarized as follows: soy products offer an economical dairy substitute for developing communities while enrichment of the protein fraction yields a high value protein concentrate with enormous economical potential in the health food industry. (Akoum *et al.,* 2006)

2.6 Membrane Technology

Membrane systems played a major role in the purification of the earliest biotechnology products. These processes adopted directly from technology that was originally developed for the blood fractionation, food, dairy, and water industries (Reis *et al.,* 2007; Kosikowski, 1986).

Over the last 2 decades, new membranes, modules, and systems have been developed specifically to meet the requirements of the biotechnology industry, which demands for higher productivity, lower cost of production, and increased in development speed (Reis *et al.,* 2007).

Membrane based separation processes are generally classified on the basis of the membrane pore size or on the type of material being processed. However, it must be emphasized that membrane pore size is not the sole basis for separation in UF processes (Ghosh, 2003).

The greatest interest of membrane process used for bioseparation has been in the application of the pressure-driven processes of ultrafiltration, microfiltration, and virus filtration (Reis *et al.,* 2007). Figure 2.1 illustrates characteristics of different pressure-driven membrane processes.

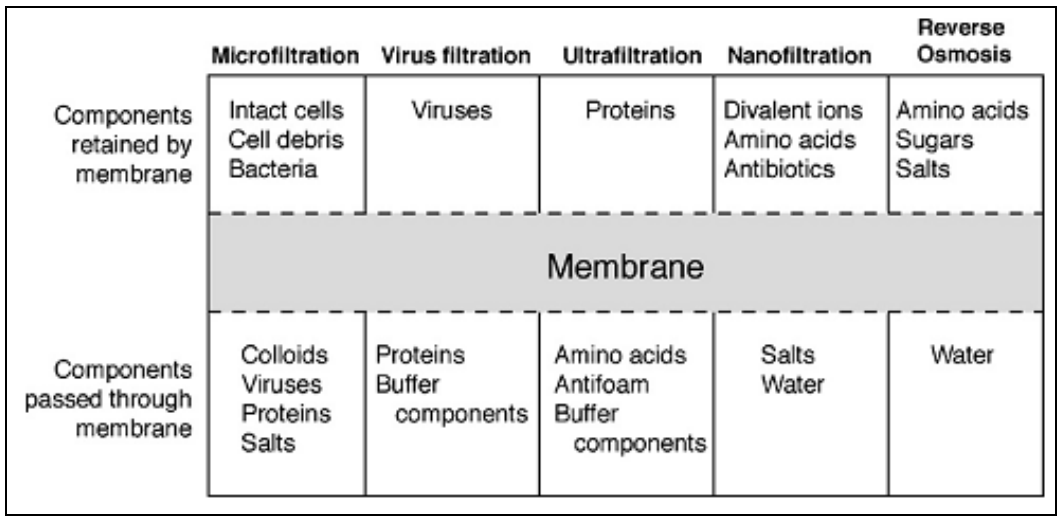

Figure 2.1 Comparison of removal characteristics of different pressure-driven membrane processes. (Reis *et al.,* 2007)

2.7 Ultrafiltration (UF) for Protein Bioseparation

Ultrafiltration (UF) is a pressure-driven membrane based separation process. UF is mainly used for protein concentration, diafiltration, clarification and fractionation. (Ghosh, 2003). UF is operated by using membrane having pore sizes ranging from 1 to 20 nm and are designed to provide high retention of proteins and other macromolecules (Reis *et al.,* 2007).

2.7.1 Protein Concentration

Protein concentration involves the removal of solvent (i.e. water) from protein solutions. On small-scale, proteins may be concentrated using different laboratory method such as vacuum evaporation and centrifugal UF. UF is the method of choice for

large-scale protein concentration. The protein-water selectivity in UF is not a real challenge, due to significant difference in molecular size.

The main issue in such operation is achieving satisfactory permeate flux, as it tends to decrease as the feed concentration increases. Also at very high concentrations, proteins tend to form gels, making it difficult to handle the. Another important issue is maintaining the activity of bioactive proteins, for example prevention of protein denaturation during processing.

2.7.2 Diafiltration

Diafiltration is a method by which low molecular weight solutes, for example salts or peptide fragments, are removed from protein solutions through UF membranes. Diafiltration is also used for buffer exchange.

As with protein concentration, the selectivity is not a major issue, on account of the significant difference in solute size. However, the transport of low molecular weight solutes can be influences by the present of proteins in the feed.

2.7.3 Protein Clarification

Protein clarification refers to the removal of particulate matter from protein solutions. The objective of membrane based protein clarification process is the efficient removal of particulate matter, along with high protein recovery. Microfiltration is perhaps more widely used for clarification processes.

2.7.4 Protein Fractionation

Techniques traditionally used for protein fractionantion in research laboratories (for example, chromatography, electrophoresis, and affinity separation) are excellently suited for purifying small quantities of proteins. However, these processes are difficult to scale-up, and this limits the scale of production. In addition to scale-up problems, techniques such as chromatography and electrophoresis require complex and expensive equipment.

Protein fractionation using UF is strongly influenced by operating and physiochemical parameters and hence such processes need to be very precisely 'fine-tuned' to achieve satisfactory level of separation. The fine-tuning exercise includes optimization of pH, salt concentration, permeate flux, and system hydrodynamics.

2.8 Mode of Separation in Ultrafiltration (UF)

Ultrafiltration (UF) process can be operated in two modes, which are dead-end filtration and crossflow filtration. These two modes are differ in their flow behaviour, and thus affecting the UF process.

2.8.1 Dead-End Filtration

Dead-end filtration, also referred to as direct flow or normal flow filtration, is used primarily for systems in which the retained components are present at very low concentration (Reis *et al.,* 2007).

This mode of filtration is commonly used in laboratories to characterize UF membranes and their separation behavior. Additionally, protein separation data from such systems are used for process scale-up (Becht *et al.,* 2008).

2.8.2 Crossflow Filtration

In crossflow filtration, the fluid to be filtered flows parallel to the membrane surface and permeates through the membrane due to a pressure difference. The crossflow reduces the formation of a filter cake and keeps it at a low level. It is possible to get a quasi-steady filtrate flow for a long time (Ripperger, *et al.,* 2002). Figure 2.2 illustrates the principle of crossflow filtration.

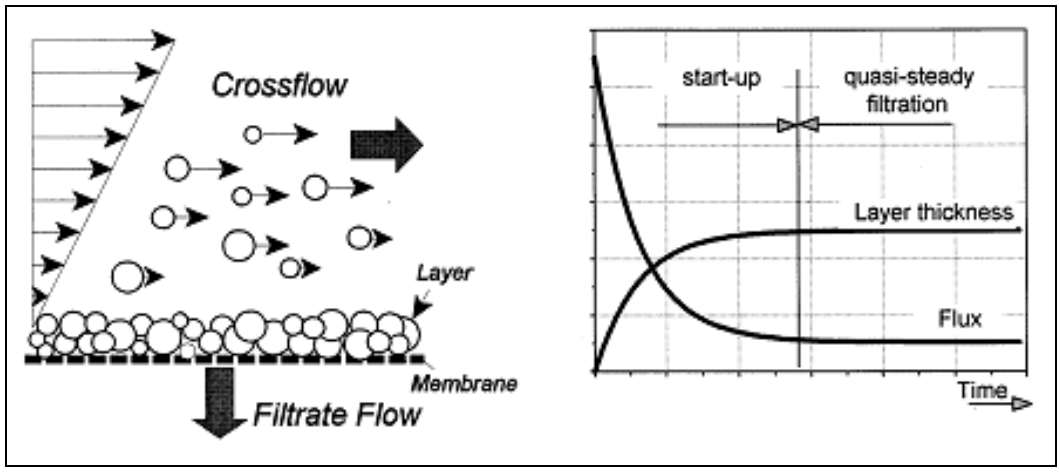

Figure 2.2 Principle of crossflow filtration (Ripperger, *et al.,* 2002)

2.9 Performance of a Membrane

A membrane is defined as a thin barrier or film through which solvents and solutes are selectively transported (Ghosh, 2003). An ideal UF membrane must have the following characteristics (Ghosh, 2003; Michaels, 1968):

a. High hydraulic permeability towards solvent
b. Appropriate sieving property (molecular weight cut-off)
c. Good mechanical durability
d. Good chemical and thermal stability
e. Compability with substances being processed
f. Excellent manufacturing reproducibility
g. Ease of manufacture

UF processes are operated in the 0-500 kPa (0-5 bar) transmembrane pressure (TMP) range. A membrane should therefore be strong enough to withstand this applied pressure. Proteins as such do not represent any challenge toward membranes from the point of view of chemical stability. However, proteins do have a strong tendency to adsorb on different types of surfaces. A good UF membrane should allow low binding.

UF relies on the ability of a membrane to act as a selective barrier, as a ‘sieve’ for macromolecular substances. This sieving property is not based on geometric considerations alone but on the combination of different effects, like molecular weight cut-off (MWCO) and hydraulic permeability.

2.9.1 Molecular Weight Cut-Off (MWCO)

Most membrane manufacturers prefer to use nominal molecular weight cut-off (NMWCO) or simply the molecular weight cut-off (MWCO), an arbitrary parameter to specify sieving properties of a membrane. (Ghosh, 2003).

This parameter defines as the molecular weight of a compound having rejection coefficient of 0.9 with respect to the membrane. In other words if a compound having a MWCO of 100 kDa is 90 percents retained by a membrane, its MWCO is designated as 100 kDa.

The concept of MWCO has been adopted with an obvious reference to the molar mass of retained macromolecules. (Zeman *et al.,* 1996). However, the retention of a compound does not depend on molecular weight alone. Therefore, the use of MWCO is arbitrary and this value is at best an approximate guide for membrane selection.

Akoum *et al.,* (2006) has investigated the feasibility of producing a soy milk fraction enriched in soybean trypsin inhibitors (STI) by using two different pore size of membrane, which are 50 kDa and 300 kDa. They found out that, the 50 kDa membrane used is well suited to the concentration of soy milk proteins with a rejection coefficient of about 98 percent and presented a higher permeate flux than the 300 kDa membrane.

2.9.2 Hydraulic Permeability

Another important indicator of UF membrane functionality is its hydraulic permeability (Zeman *et al.,* 1996). The hydraulic permeability of a membrane is depending on the percentage porosity, the pore size distribution and the membrane

thickness. The higher the hydraulics permeability, the higher the potential productivity. (Ghosh, 2003).

Usually it is expressed as a volume flow of a liquid through a unit area of membrane at some defined TMP, and it is measured in units of velocity per unit of pressure. For water, a typical range of UF fluxes is about 6.80×10^{-6} to 6.80×10^{-2} cm/(sec kPa) (Zeman *et al.,* 1996).

The hydraulic permeability can be determined by filtering pre-filtered deionised (DI) water through the membrane at different TMP. The permeate flux (filtration rate per unit membrane surface area) is plotted against the applied TMP and the slope of the straight line thus obtained gives the hydraulic permeability of the membrane (Ghosh, 2003). If the permeate flux versus TMP plot in DI water filtration is not linear, the membrane can be assumed to be susceptible to pressure induced deformation.

2.10 Operating Factors in Ultrafiltration (UF) Separation Process

In Ultrafiltration (UF) separation process, there are operating factors that need extra concern before the separation occurs. These factors are:

a. Transmembrane pressure (TMP)

b. Permeate Flux

c. Retention Ratio

2.10.1 Transmembrane Pressure (TMP)

The main driving force in the UF process is transmembrane pressure (TMP), which range from 0 to 500 kPa (0 to 5 bar). The TMP can be expressed by the following equation:

$$TMP, \Delta P = \frac{\left(P_{feed} + P_{retentate}\right) - P_{permeate}}{2} \quad (2.1)$$

When TMP is applied to the system, the bulk solution containing solute molecules is forced through the pores of membrane. During this process, the solute molecules are being carried by the solvent towards the membrane, and in certain cases through the membrane. Solute molecules may be fully transmitted, partially transmitted, or totally retained (or rejected) by the membrane. (Ghosh, 2003).

The effect of varying TMP on the microfiltration fouling has been studied by Thomassen *et al.* (2005) on a model beer. They found out that an increase in TMP resulted in a reduction in transmission of biological components through the membrane. Higher TMPs lead to an increase in the proportion of biological compounds retained by the membrane.

2.10.2 Permeate Flux

Permeate flux, J_v is defined as volume or mass of liquid crossing the membrane per unit area per unit time. It is usually expressed in liter/m^2/hr or simply LMH by the following expression:

$$Permeate\ flux,\ J_v\ (LMH) \quad = \quad \frac{Permeate\ flow\ rate\ (mL/\min)}{Membrane\ area\ (m^2)}\ x\,0.06 \qquad (2.2)$$

Permeate flux represents the productivity of a membrane separation process. It depends on the properties of membrane, the TMP, the system hydronamics, the protein concentration in the feed and the properties of solvent and the protein (Ghosh, 2003).

It is often observed that the permeate flux in an UF process does not increased linearly with TMP beyond a certain point. As the pressure is increased even further, the permeate flux levels off. A still further increase in TMP may even lead to a decline in permeate flux.

The pressure range in which the permeate flux increases with increase in TMP is referred to as the pressure dependant region. The range where the increase in pressure does not increase the permeate flux is referred to as the pressure independent region. The permeate flux in the pressure independent region is referred to as the 'limiting flux' (Ghosh, 2003).

2.10.3 Retention Ratio

The retention ratio, R_{et} is a convenient indicator of the relative amounts of protein molecules on either side of the membrane (Thomassen *et al.,* 2005). The

retention of protein molecules by the membrane depends on steric, hydrodynamic, thermodynamic and electrostatic effects (Ghosh, 2003).

Typically, for protein molecules having size equal with the membrane pore rating, R_{et} is greater than 0.9. Sieving coefficient, S (S = 1- R_{et}) is contradict to R_{et}, as the value of S of protein molecules through the membrane does not exceed 0.1. (Zeman *et al.,* 1996).

As mentioned earlier, there is a decline in permeate flux as the TMP increase. This is due to the build-up of rejected protein molecules near the membrane surface. The rejected protein molecules, which accumulate near the membrane surface, result in a transmembrane concentration difference.

The retention ratio for protein molecules can be calculated through the application of following equation:

$$R_{et} = 1 - \frac{C_p}{C_o} \qquad (2.3)$$

R_{et} is retention ratio within the retentate of specific solute (dimensionless), C_p the concentration of solute in the permeate (mg l^{-1}), and C_0 the concentration of solute in the retentate(mg l^{-1}). If the value of R_{et}=0, it indicates that all particles are transmitted through the membrane, and thus the separation process can be considered as fail. However, if the value of R=1, it means that the separation process having a complete retention, and the desired product is smaller particles that has been transmitted to permeate.

Thomassen *et al.* (2005) also has studied on the retention ratio of dextrin and bovine serum albumin (BSA) of a model beer. Their results indicated that increases in both time and TMP result in an increase in both components retention ratio.

2.11 Protein Concentration

The measurement of total protein at a given step may be important in the following circumstances (Scopes, 1982):

a. when fractionation step is critically dependant on the protein concentration
b. when it is necessary to know whether a particular step has really removed much unwanted protein
c. to test the specific activity at the final preparation to see if it has reached the maximum value expected for the pure protein.

Some of the available and most widely used methods for measuring proteins are Biuret Method and Lowry Method. Each of these has particular advantages and disadvantages. The only 'perfect' way to measure protein is by dry weight determination, but it requires the sample to be sacrificed for the measurement. Accurate measurements can only be made for pure proteins, after those pure proteins have been standardized for that method against a dry weight determination.

2.11.1 Biuret Method

This method involves a strongly alkaline copper reagent which produces a purple coloration with protein. It gives a fairly accurate measurement since there is little variation in color yield from protein to protein. This is because the copper reagent reacts with the peptide chain rather that side groups (Scopes, 1982).

Ammonia interferes by complexing with copper, so ammonium sulfate fractions do not give accurate results. A greater sensitivity of the biuret reaction is achieved by

observing the copper-protein complex not at 540 nm, but at 310 nm in the near-ultraviolet (Scopes, 1982).

The principle reason why it is not widely used in research is its low sensitivity; several milligrams of sample must be sacrificed for a reliable measurement.

2.11.2 Modified Lowry Method

This method procedure is particularly sensitive because it employs two color-forming reactions. It uses the Biuret reaction in which Cu^{2+} (in the presence of base) reacts with the peptide bond to give a deep blue color (Scopes, 1982).

In addition, Folin-Ciocalteu chemistry, in which a complex mixture of inorganic salts reacts with tyrosine and tryptophan residues to give an intense blue-green color, is also used. The combination of the two reactions gives an assay that is more sensitive that either reaction alone (Scopes, 1982).

Many modifications have been reported, mainly to avoid interference by specific components. Unfortunately, many of the compounds used in enzyme purification interfere with the reaction, and different modifications are required to overcome each problem.

But since it is a sensitive method giving a good color with 0.1 mg ml^{-1} of protein or less, interfering compounds are often diluted out to levels where their effects is insignificant.

CHAPTER 3

METHODOLOGY

3.1 Introduction

Transmembrane pressure (TMP) is the main applied driving force in the membrane separation processes (Ghosh, 2003). The TMP will affect the performance of the membrane by means, the transmission of biological components through the membrane and the fouling layer thickness (Thomassen *et al.,* 2005). By varying TMP, various qualities of materials being retained and being permeated can be obtained. Therefore, through this research project, the separation process were done using different TMP but at the same conditions, in order to determine which TMP value can allow the membrane to perform better.

Samples from commercial soy milk were prepared by differential centrifugation to remove large solid particles, before being introduced to the ultrafiltration (UF) system of Kvick Cross Flow. 50 kiloDalton (kDa) membrane was used, while operating parameters like pH and temperature in the feed tank were held constant throughout the experiment. TMP values were manipulated, and volume of permeate samples were collected every 60 seconds for seven minutes. Flux decline data was obtained by measuring permeate flux from the permeate volume collected. The permeate and retentate samples were assayed to determine the composition of protein by using Modified Lowry Assay method. Further data manipulation has enabled retention ratio to

be calculated for each experiment. The cleaning process of the membrane was done after each experiment, and the water flux determination was performed to check the effectiveness of the cleaning process on the membrane. Figure 3.1 summarizes the methodology used in this research project.

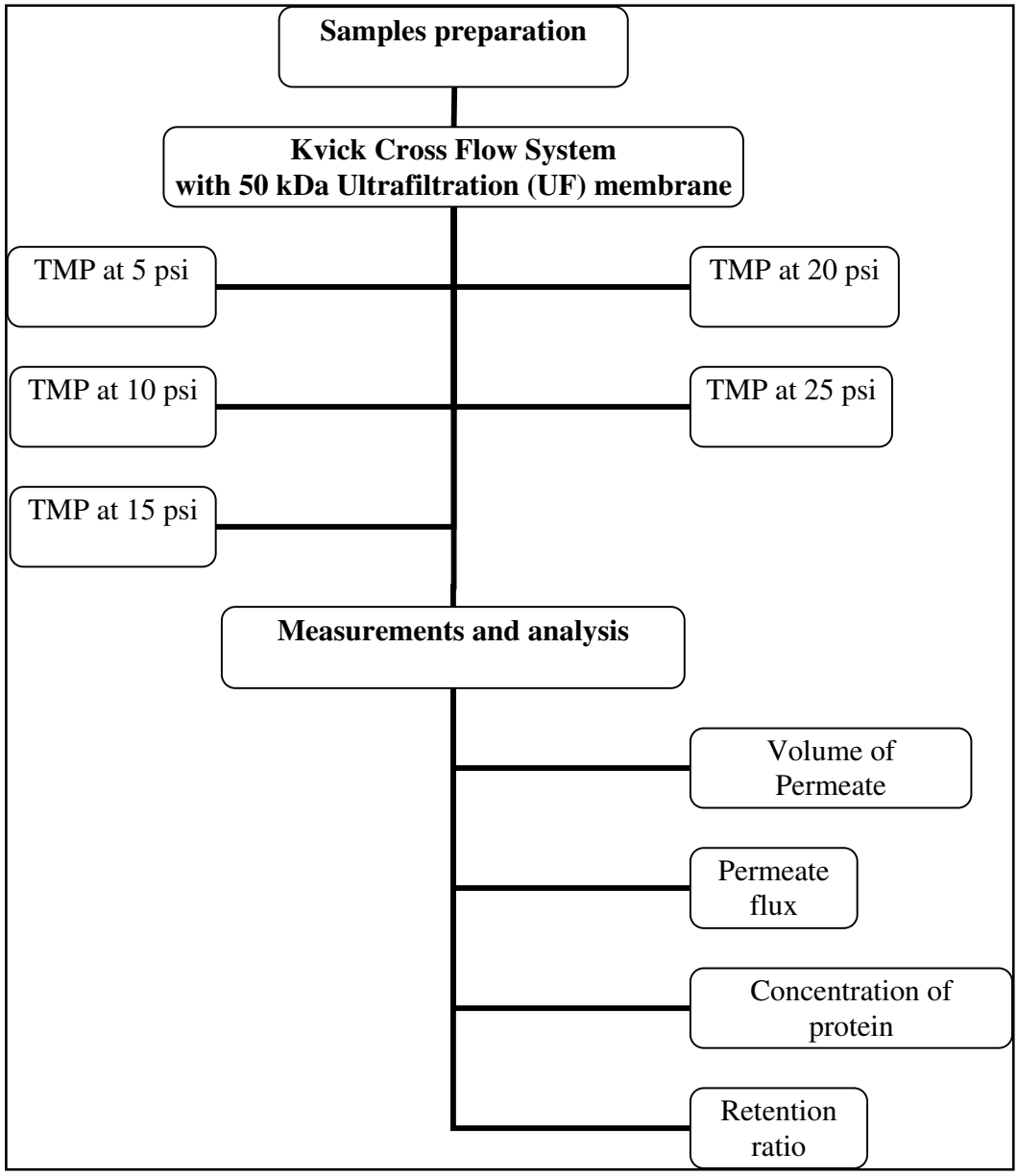

Figure 3.1 Schematic diagram of research project methodology

3.2 Samples Preparation

The samples of soy milk were obtained from the commercial soy milk, for this case, the Green Love's Soy Talk soy milk. This commercial brand was chosen because of the availability in the market that allows the samples to be purchased in abundance.

The comparison of nutritional fact of various soy milks available in Malaysia market is attached in the Appendix A. Based on the nutritional facts, it can be seen that for this commercial brand, the protein constituent 2.0 g in 100 mL of the soy milk. Thus, the initial concentration of the protein in the sample should be 0.02 g/mL.

The pH of the samples was constant throughout the experiment at 6.87. This followed a statement from Zhang *et.al.*, 2005, which indicated that there is no significant differences in pH value throughout the UF experiment. In addition, the value of pH which was stable indicates the absence of bacterial development (Akoum *et al.,* 2006). All samples were run through the Kvick Lab Crossflow system at room temperature.

Since the soy milk is actually a turbid and colloidal solution, which has high solid content that can induce strong membrane fouling, a pre-treatment step to the sample must be done. This step was important to prevent the solid particles in the soy milk from clogging up the membrane. Differential centrifugation method was used, where the soy milk was centrifuged at 8000 g and 20°C for 15 minutes.

Based on Ono *et.al.*, 1991, for a raw soymilk being centrifuged at 8000 g and 20 °C for 30 minutes, particles of more than 200 nm in size would be pelleted. In consideration of the samples being used were commercial soy milk, and also limitation of equipment for centrifugation, the centrifugation time for all samples in this research project were reduced to 15 minutes. However, a precaution step was taken, as the supernatant from the centrifugation process was filtered through a 201 Double Rings

Filter paper, while the pellet was discarded, producing a homogenous soy milk solution, free from large particles.

3.3 Kvick Lab Crossflow System

The UF system used in this research project was Kvick Lab Crossflow System from Amersham Biosciences. This system designed with the 2.5 liter reservoir, pump, pressure gauges, cassette holder, piping, and fittings, make the system quick to set up and easy to use. The membranes used was a flat sheet membrane, made of polyethersulfone (PES), which had 0.11 m^2 (1.2 ft^2) of membrane surface area and 50 kDa molecular weight cut-off (MWCO).

50 kDa pore size of membrane was chosen because at this pore size, all of the solubilised proteins are recovered. Larger membrane pore size like 100 kDa is inadequate to be used, as smaller proteins would permeate the membrane, thus lower the protein yields (Alibhai *et al.,* 2006). In addition, this pore size of membrane allowing soybean trypsin inhibitor (STI) of 8 kDa (Bowman-Birk inhibitor) and 20 kDa (Kunitz inhibitor) in size to be recovered in permeate for further analysis.

3.3.1 Transmembrane Pressure (TMP)

Transmembrane pressure (TMP) can be calculated by applying the Equation (2.1):

$$TMP, \Delta P = \frac{\left(P_{feed} + P_{retentate}\right) - P_{permeate}}{2} \quad (2.1)$$

In this research project, the TMP values of the UF process were varied accordingly to 5, 10, 15, 20, and 25 psi by tuning both feed pressure and retentate pressure to desired TMP.

As the UF equipment of this research project did not have a pressure gauge at the permeate line, permeate pressure for above equation is assumed to be zero. This assumption is made based on the user manual of the equipment used. The feed pressure was tuned by varying the rotation speed of feed pump, while the retentate pressure was tuned by turning the knob at the retentate line.

3.3.2 Rinsing the Cassette from the Storage Solution

Before opening new cassette bag, safety glasses, gloves and lab coat were worn. This is because the cassette is stored in a solution containing 0.2 N of sodium hydroxide (NaOH) and 22 percent glycerin. It was opened over a sink and excess solution from the cassette was drained with high unity of water. Both cassette and its cassette bag were rinsed with large amount of water to minimize the hazardous effect of its storage solutions.

3.3.3 Installation of Membrane Cassette

The silicon gasket was wet and be placed against the manifold by aligning the holes. At Kvick lab holders, a cassette was installed, with integral gasket facing the backing plate. The spacers was added slides up to the backing plate of the cassette and each nut was tightened alternately ¼ turn at a time with a torque wrench to 180 lb / in.

3.3.4 The Cleaning and Storage of the Cassette

The cassette was cleaned by using 0.5 M of NaOH solution, followed by rinsing and backwash process with deionized (DI) water. After completing the cassette cleaning process, the cassette was stored in DI water before next usage.

3.4 Measurements

After allowing the samples to flow through the Kvick Cross Flow System with 50 kDa UF membrane, there are several measurements done to achieve the objective of this research project. These include:

a. Permeate flux
b. Water flux after separation
c. Concentration of protein
d. Retention ratio

3.4.1 Permeate Flux

Volume of permeate was collected at 60 seconds interval for seven minutes. Graduated cylinder was used to meet this purpose. The permeate flow rate was calculated by using the following equation:

$$Permeate\ flow\ rate\left(mL/\min\right) = \frac{Volume\left(mL\right)}{Time\left(\min\right)} \quad (3.1)$$

Permeate flux, J_v is the value of permeate flow rate per unit area of membrane, was calculated by the application of Equation(2.2):

$$Permeate\ flux,\ J_v\ (LMH) = \frac{Permeate\ flow\ rate\ (mL/\min)}{Membrane\ area\ (m^2)}\ x\,0.06 \qquad (2.2)$$

The permeate flux was plotted against TMP to obtain the flux decline data at certain TMP value.

3.4.2 Water Flux after Separation

The water flux measurement after each cycle is important to determine the effectiveness of the cleaning process as well as to observe defection on the cassette used.

Water flux obtained before experiment started, when the cassette was new and defect-free, has become the basis value for the water flux determination. The feed tank was filled with water, and the system was allowed to run at constant TMP of 10 psi. The water permeate was collected using graduated cylinder for 60 seconds while the temperature in the reservoir is recorded. Then, the initial water permeate flux is calculated and this value was normalized to 20°C and 1 psig, in reference to values tabulated in Table C.2 of Appendix C.

After the experiment using a sample being done and the cassette was cleansed and rinsed, the feed reservoir is filled up again with water. The system is again allowed to be operated at 10 psi, and the water permeate flux was determined and normalized to 20°C and 1 psig.

A comparison between the water flux before and after the cycle was made and the percentage of comparison is calculated. The use of the membrane should be stopped if the percentage of the comparison is less than 60 percent.

In this research project, the percentage of the comparison is around 50 percent to 60 percent throughout the whole experiments. Due to unavailability of alternative membrane, the membrane is kept being used in this experiment, by assuming that the cleaning process is adequate at 50 percent. This cleaning and re-used process after each experiment is a replication of industrial filtration condition.

3.4.3 Concentration of Protein

Modified Lowry Method was chosen to be used in this research project. This is due to the sensitiveness of this method compared to others. A standard curve; Calibration Curve of Bovine Serum Albumin (BSA) was used as a basis for protein quantification.

Lowry solution is the result of mixing reagent A to B in a 50:1 ratio. Reagent A was prepared by dissolving 20 g of sodium carbonate and 4 g of sodium hydroxide in 1 liter distilled water. This reagent is then kept refrigerated. Reagent B was prepared by dissolving 2.5 g of copper sulfate ($CuSO_4.5H_2O$) and 5 g of sodium citrate in 1 liter distilled water. The bottle was wrapped with aluminium foil to avoid discolorization and kept refrigerated. On the other hand, the Folin-Ciocalteu solution is prepared by dilution of the stock with distilled water in 1:1 ratio.

Due to various turbidity of samples obtained and also limitation of the reading range of UV-Vis Spectrophotometer, various amount of dilution factor were used in this research project. The values of all dilution factors will be presented in Chapter 4.

0.2 mL of the diluted sample was added and mixed well with 1 mL of Lowry solution and was leaved at room temperature for 10 minutes. After that, 0.1 mL of Folin-Ciocalteu solution prepared earlier was added and mixed well, and was leaved for 30 minutes, also at room temperature.

The Optical Density (OD) of the sample and assay mixture was measured against blank at 750 nm by using UV-Vis Spectophotometer. The value of the OD read was compared with the Calibration Curve of BSA prepared earlier to get the value of the concentration of protein in the samples. These procedures were repeated for each samples of permeate and retentate.

3.4.4 Retention Ratio

After obtaining the concentration of permeate (C_p) and retentate (C_o), the retention ratio (R_{et}) for protein molecules on the membrane was calculated through the application of Equation (2.3):

$$R_{et} = 1 - \frac{C_p}{C_o} \tag{2.3}$$

The retention ratio was plotted against TMP to get the idea of how much protein being retented on the membrane by particular TMP.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

This research project involved manipulation of data, to get the idea on the performance of the membrane during the ultrafiltration (UF) process. The performance of a membrane under influence of transmembrane pressure (TMP) can be seen from the measurements of the permeate flux, concentration of protein transmitted and retention ratio by the membrane.

4.2 Results

There are four types of graphs plotted in this research project, and will be discussed later in this chapter. The graphs are:

a. Permeate flux versus TMP
b. Permeate flux versus time
c. Concentration of protein transmitted versus TMP
d. Retention ratio versus TMP

4.2.1 Permeate Flux Analysis

Analysis on the permeate flux is very important in any membrane related processes. Permeate flux basically depends on the properties of the membrane, the TMP, the system hydronamics, the protein concentration in the feed and the properties of solvent and the protein (Ghosh, 2003). As other parameters except for TMP were held constant, thus, permeate flux analysis can give the insights on the effect of the TMP on the UF membrane performance of soy milk.

4.2.1.1 Permeate Flux versus TMP

Figure 4.1 shows the graph of permeate flux at various TMP values. It can be seen that the permeate flux is increasing, with the increasing of TMP. As stated by Ghosh, 2003, the permeate flux is often being observed to be increased in non-linearly manner with TMP. The result seems to obey the statement.

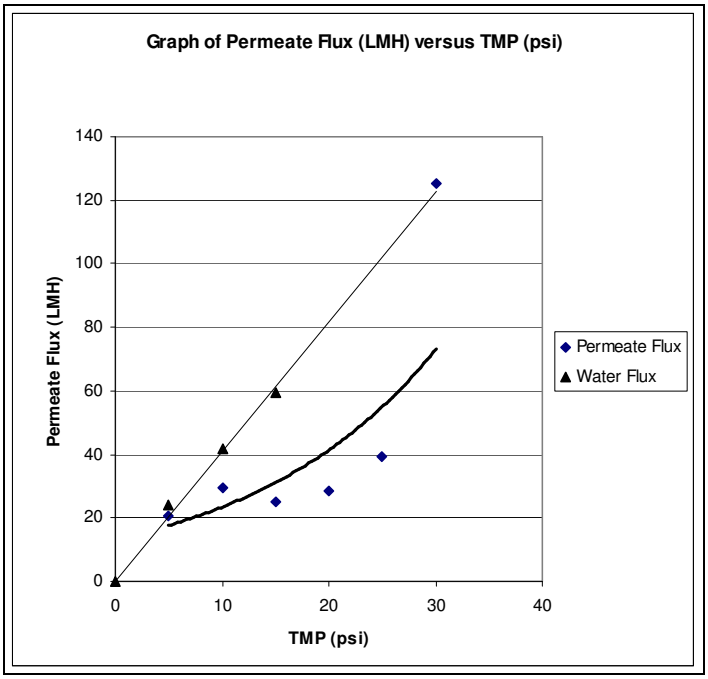

Figure 4.1 Graph of Permeate Flux versus TMP

TMP is a driving force for the soy protein to be transmitted through the membrane. Differences in TMP used make the soy protein encounter different pressure forces. At lower TMP values, the soy protein is difficult to pass through the membrane, while at higher TMP values, the soy protein can easily transmitted through the membrane.

A comparison of the permeate flux of soy protein has been made with the water flux. It can be seen that at certain TMP value, the permeate flux of soy protein is much lower compared to water flux. This is mainly due to the fact that the water has much lower viscosity compared to soy milk. As a result, water flowed through the membrane at higher flux.

However, at TMP value of 30 psi, the permeate flux of soy milk has reached nearly the value of the water flux. This indicates that, despite of the differences in the viscosity of water and soy milk, TMP of 30 psi gave 'dilution effect' to the soy milk. This makes the soy milk transmitted through the membrane at high flux, as well as water.

4.2.1.2 Permeate Flux versus Time

Graph of permeate flux of soy protein at various TMP values over time is illustrated in Figure 4.2. From that particular graph, it is seen that permeate flux gives different values when TMP is changed. At TMP values of 5 and 10 psi, the permeate flux decreased over time. When TMP of 15 psi being applied, the permeate flux give constant value over time. For TMP values of 20 and 25 psi, the permeate flux increased over time, and being constant after 300 seconds.

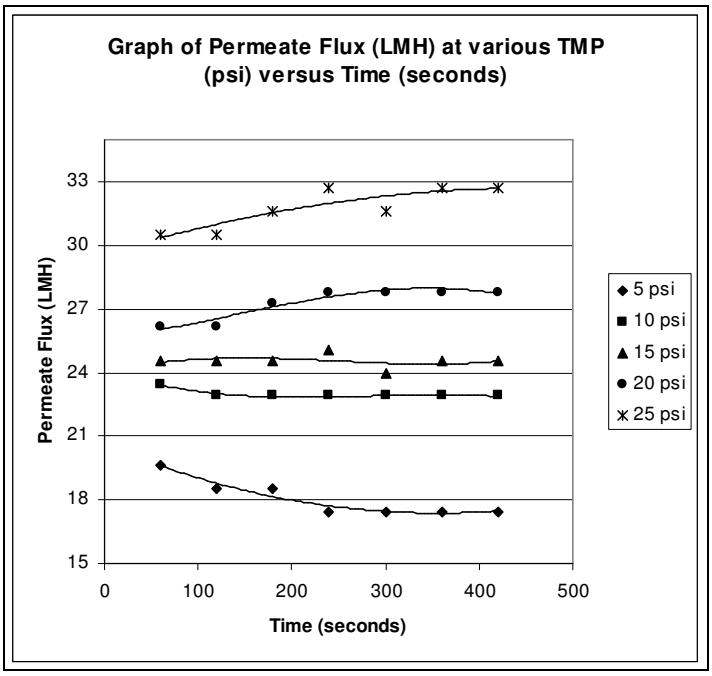

Figure 4.2 Graph of Permeate Flux at various TMP versus Time

The permeate flux over time for 5 psi and 10 psi seem to be contradicted with the statement of Ghosh, 2003, mentioned earlier. It can be seen that the permeate flux is higher at the beginning of the process, and keep decreasing until it became constant at 250 seconds. This phenomenon is caused by the insufficient TMP used to make the soy milk permeated the membrane. The flux is relatively low compared to other, hence a higher TMP value needed to facilitate the transportation of soy milk across the membrane.

For 15 psi, a different trend occurred whereby a constant reading was easily achieved by 200 seconds. This is due to the easy penetration of soy milk through the membrane, which indicates that the TMP being applied is sufficient.

In contrast to what have happened to the permeate flux in lower TMP values, at TMP values of 20 and 25 psi, the flux seem to be increased over time and following the statement from the reference. However, after a short time, the permeate flux become constant and flowing at steady state. This indicates that, the membrane has been fouled

by the soy milk particles. Further increases beyond these TMP values may lead to a decline in permeate flux.

At this point, initial comparison on membrane performance under influence of TMP shows that the efficient TMP value in order to ultrafiltrate soy protein is in the range of 15 psi and 30 psi. This is because, at stated TMP range, the permeate flux shown an efficient behavior, where it gives higher flux value in short time. However, to get the most efficient TMP values, further comparison on other parameters should be done, as will be discussed in the next part.

4.2.2 Concentration of Protein Transmitted Analysis

As UF of soy milk at 50 kDa membrane can recover soybean trypsin inhibitors (STI) in permeate, it is desired to evaluate the concentration of protein that can be transmitted through the membrane that will affect the purity of recovery. The protein concentration measurement in permeate is important to determine which TMP value is selective on soy protein.

4.2.2.1 Bovine Serum Albumin Calibration Curve

It is widely acceptable to use readily available proteins such as bovine serum albumin (BSA) or gamma globulin as protein standards. This is because, it is difficult to attain a protein standard with similar properties to the sample being analyzed. In this research project, BSA was used as standard due to its availability and low cost.

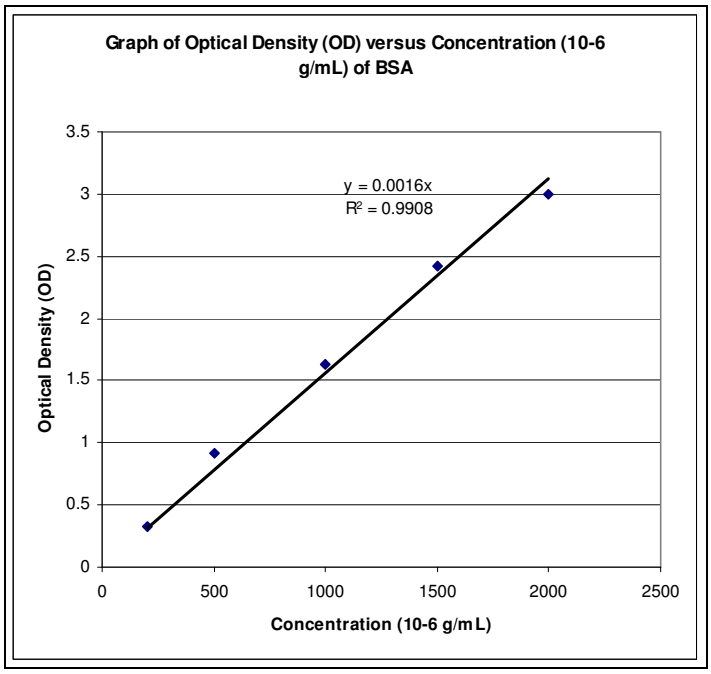

Figure 4.3 Calibration Curve of BSA protein standard

Graph of Optical Density (OD) versus Concentration of BSA, will be used later as Calibration Curve of BSA protein standard throughout this research project is illustrated in Figure 4.3. This calibration curve is very important in the protein analysis step, as every OD measurement from samples will be compared with this graph to get the value of protein concentration in the sample. Due to its importance, this graph should be reliable and accurate. Several trials were done in order to get closer regression to 1. The graph above has the regression of 0.9908, thus it is acceptable to be used in this research project.

4.2.2.2 Concentration of Protein Transmitted versus TMP

The graph of concentration of protein transmitted versus TMP is illustrated in Figure 4.4. Based on the graph, it can be seen that the concentration of protein being transmitted through membrane increased as the TMP increased. Theoretically Alibhai *et*

al., 2006 has stated that for a 50 kDa pore size of membrane, all solubilized protein are retained. Due to several TMP that have been used, the membrane has lost its ability to retain the protein. As a result of that, some soy protein has permeated.

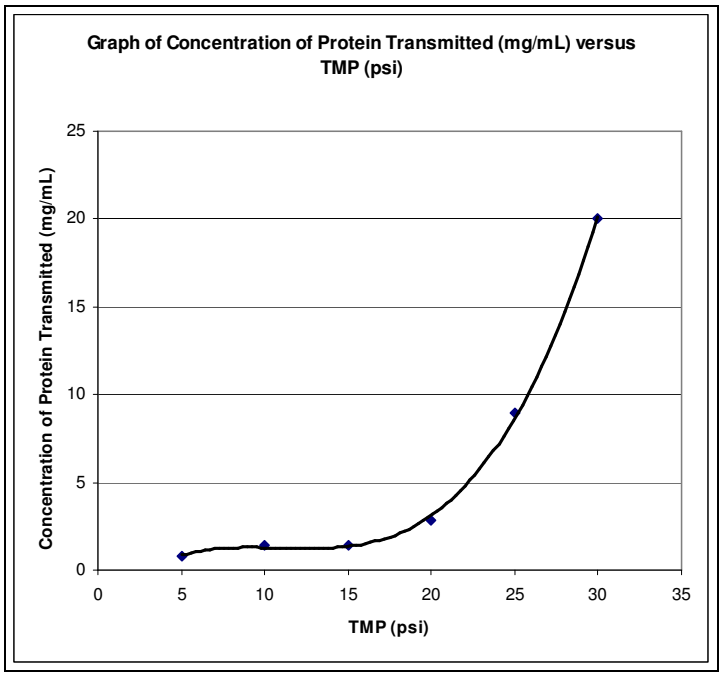

Figure 4.4 Graph of Concentration of Protein Transmitted versus TMP

Since turbidity of permeate were not measured in this research project, physical observation shows that the turbidity of permeate increased as TMP exceed 20 psi. This is due to more soy protein being transmitted through the membrane. This also shows that exceeding 20 psi, the membrane is less selective to the soy protein.

In particular to protein concentration analysis run under several TMPs, it is seen that the best TMP value is in the range of 15 to 20 psi. In stated range, the permeate flux is high and the soy protein selectivity is good. Further comparison will be made in the next part, in order to investigate which TMP value gave the best membrane performance in order to ultrafiltrate soy protein from soy milk.

4.2.3 Retention versus TMP

The retention ratio, R_{et} is a dimensionless indicator that can determine the relative amounts of protein molecules on either side of the membrane (Thomassen *et al.,* 2005). R_{et}=0, will indicates that all particles are transmitted through the membrane, while the value of R=1 means that the separation process having a complete retention. Figure 4.5 illustrates the retention ratio of protein versus TMP.

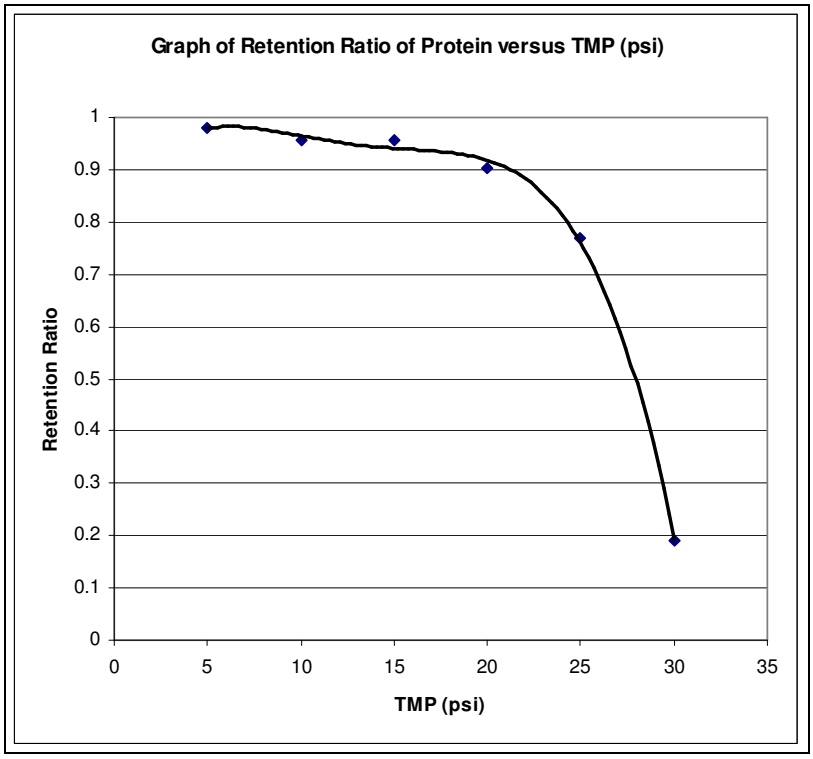

Figure 4.5 Graph of Retention Ratio of Protein versus TMP

Based on the graph above, it can be seen that the retention ratio is decreased with the increasing of TMP. Significant reduction of retention ratio can be seen when the TMP is increased to higher value, where the value falls from 0.9 to 0.76. This indicates that the separation is failed as soy protein can be transmitted freely through the membrane due to high pressure applied. The value of retention ratio for TMP lower than 20 psi is acceptable, as it still retained 90 percent of soy protein on the membrane.

Based on the retention ratio calculation, it is observed that the membrane can withstand the soy protein by 90 percent at TMP of lower than 20 psi. In conclusion to all parameters used in the analysis process, the effective TMP range is between 15 and 20 psi. This is due to high permeate flux, good selectivity of membrane on soy protein and 90 percent soy protein retention on the membrane.

4.3 Discussions

As this research project is deeply concern in the performance of the membrane, several precaution steps were taken in order to minimize the possibility of defection to the membrane used. All samples of soy milk being used were centrifuged at 8000g, 20 °C for 15 minutes, in order to minimize clogging. Based on studies of Ono *et.al.*, 1991, this step can removed relatively large protein particles by the size of 200nm.

Moreover, the cleaning procedures were strictly executed. As the equipment for this research project were also used by others with various of samples, it is very important to minimize the effects of other samples on the protein measurement of soy milk samples. Deionized (DI) water has been used for all cleaning procedures. The procedures include the rinsing of the system, sanitizing the system with 0.5 M Sodium Hydroxide (NaOH), and rinsing the system again, before the samples are allowed into the system.

Water flux determination was done from the very beginning of the membrane usage. The performance of the membrane was decreased to 50 percent after the first sample being introduced. Comparisons of each water flux after each cleaning and backwash process were made, and the performance of the membrane remains in the range of 50 to 60 percent. Common understanding in several journals stated that membrane should be discarded if the membrane performance reduces below 60 percent.

Due to economic inability to purchase new membrane at all times, the performance of 50 percent was accepted.

This research project also experienced some problems and limitations, mainly from the equipments being used. The performance and reliability of equipments such as centrifuge, chiller and also the Kvick Cross Flow system have indirectly affected the results of this research project.

As mentioned earlier, centrifuge equipment is needed in the sample preparation step to separate large protein particles from the samples. However, the centrifuge with large capacity has broken, and only small centrifuge with capacity of 6x40mL is available to be used. Approximately, 225 centrifugation processes has been done throughout this research project by using the small centrifuge. This indirectly makes the separations processes are not constant as the centrifugation step need to be done batch by batch.

The samples need to be chilled in a chiller to prevent any microorganisms from contaminating the samples after the centrifugation process. The lagging in the centrifugation step have made each sample to have different residence time in the chiller. This might affect the results in term of protein content in the sample.

In Kvick Cross Flow System, the membrane has to be fastened to its cassette by using nut and bold at 180 lb/in pressure. However, there have been cases where the fastener is failed to fasten the cassette. Alternative method has been used, which is by using G-clamp without any pressure indicator. There are some leakages of samples being observed which contribute some minor effects on the UF process itself.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.3 Conclusions

In order to fractionate soy protein from soy milk by using 50 kiloDalton (kDa) membrane; the effective range of transmembrane pressure (TMP) that should be applied to the ultrafiltration (UF) process is between 15 to 20 psi. This is the summary of the results from all parameters being tested at various TMP; the permeate flux, the concentration of soy protein transmitted and also the retention ratio. In this range of TMP, the permeate flux of soy protein is high; between 25 and 30 LMH, the concentration of soy protein transmitted is between 1.38 and 2.85 mg/mL and the retention ratio by the membrane is approximately 90 percent.

5.4 Recommendations

There are some improvements that can be done to this research project in order to improve the results. Samples preparation is very crucial, as it will effect directly to the protein content in the samples. Fresh centrifugated samples, which were not stored in the chiller, can give better results. In addition, instead of reducing the residence time of centrifugation process by half like what has been done in this research project,

improvement can be made by directly followed the method being used by Ono *et al.,* 1991. Based on the reference, this step will remove approximately 3.9 percent of soy protein with 200 nm size. This will contribute to less membrane fouling and hence, give a better membrane performance.

Moreover, further research can be done to widen the scopes of this research project. As stated by Akoum *et al.,* 2006, soybean trypsin inhibitors (STI) can be recovered in permeate of soy milk UF using 50 kDa membrane. This STI can be used in medical field and have high potential value. Further research can be done by utilizing the suggested TMP range achieved in this particular research project, to identify and quantify the STI in the soy milk. Studies from Hernández-Ledesma *et al.,* 2009 can be used as a basis for future research.

LIST OF REFERENCES

Akoum O., Richfield D., Jaffrin M. Y., Ding L.H., and Swart P. (2006). Recovery of trypsin inhibitor and soy milk protein concentration by dynamic filtration. *Journal of Membrane Science.* 279, 291–300.

Akoum O., Richfield D., Jaffrin M. Y., Ding L.H., and Swart P. (2006). Recovery of trypsin inhibitor by soy milk ultrafiltration using a rotating disk system. *Desalination,* 191, 438–445.

Alibhai Z., Mondor M., Moresoli C., Ippersiel D. and Lamarcheb F. (2006). Production of soy protein concentrates/isolates: traditional and membrane technologies. *Desalination.* 191, 351–358.

Becht N.O., Malik D.J., and Tarleton E.S. (2008). Evaluation and comparison of protein ultrafiltration test results: Dead-end stirred cell compared with a cross-flow system. *Separation and Purification Technology,* 62, 228–239.

Dunne, L. J. (2002). *Nutrition Almanac*. (5th ed.) (pp. 212). United States of America: McGraw-Hill.

Fournier, D. B., Erdman, J. W., & Gordon, G. B. (1998). Soy, its components, and cancer prevention: A review of the in vitro, animal and human data. *Cancer Epidemiological Biomarkers Preview,* 7, 1055–1065.

Furukawa T., Kokubo K., Nakamura K. and Matsumoto K. (2008). Modeling of the permeate flux decline during MF and UF cross-flow filtration of soy sauce lees. *Journal of Membrane Science*. 322, 491–502.

Ghosh R. (2003). *Protein Bioseparation Using Ultrafiltration: Theory, Applications and New Developments*. London: Imperial College Press.

Ghosh R. (2006). *Principles of Bioseparations Engineering*. Singapore: World Scientific Publishing Co Pte. Ltd.

Hernández-Ledesma B., Chia-Chien Hsieh C.C., and Lumen B.O.D. (2009). Lunasin and Bowman-Birk protease inhibitor (BBI) in US commercial soy foods. *Food Chemistry*

Kumar N.S.K. Yea M.K. and Cheryan M. (2004). Ultrafiltration of soy protein concentrate: performance and modelling of spiral and tubular polymeric modules. *Journal of Membrane Science.* 244, 235–242.

Malaki A.N., Tosh S., Poysa V., Woodrowc L., and Corredig M. (2008). Physicochemical characterization of soymilk after step-wise centrifugation. *Food Research International,* 41, 286–294.

Ono T., Myoeng Rak Choi M.R., Ako Ikeda A., and Odagiri S. (1991). Changes in the Composition and Size Distribution of Soymilk Protein Particles by Heating. *Agric. Biol. Chem.,* 55 (9), 2291-2297.

Reis R.V. and Zydney A. (2007). Bioprocess membrane technology. *Journal of Membrane Science.* 297, 16–50.

Ripperger S. and Altmann J. (2002). Crossflow microfiltration – state of the art. *Separation/Purification Technology*. 26, 19–31.

Scopes, R. K. (1994). *Protein Purification: Principles and Practice.* (3rd ed.) (pp. 44 - 49). United States of America: Springer.

Thomassen J.K., Faraday D.B.F., Underwood B.O. and Cleaver J.A.S. (2005). The effect of varying transmembrane pressure and crossflow velocity on the microfiltration fouling of a model beer. *Separation and Purification Technology*. 41, 91–100.

Zeman, L. J., and Zydney. A. (1996*). Microfiltration and ultrafiltration: Principles and Applications.* (pp. 12 – 16). New York: Marcel Dekker, Inc.

Zhang H., Li L., Tatsumi E. and Isobe S. (2005). High-pressure treatment effects on proteins in soy milk. *Lebensm.-Wiss. u.-Technol.* 38, 7–14.

APPENDIX A

Nutritional Facts of Various Commercial Soy Milk

Table A1: Comparison of Nutritional Facts of Various Commercial Soy Milk Available in Malaysia Market

	Per 100 mL		
Nutrients	**Green Love's Soy Talk**	**Yeo's**	**Season's**
Energy	54.4 Kcal	54 Kcal	63 Kcal
Carbohydrate Total Sugars	9.8 g 7.8 g	8.9 g 7.6 g	10.4 g 8.7 g
Protein	**2.0 g**	**2.0 g**	**2.1 g**
Total Fat	0.8 g	1.2 g	1.4 g
Concentration of protein (wt/v)	**2.0**	**2.0**	**2.1**
Percentage (%) of protein over other nutrients	**9.8**	**10.2**	**9.3**

APPENDIX B

Bovine Serum Albumin (BSA) Calibration Curve

Table B1: Experimental Data of Bovine Serum Albumin Calibration Curve

Concentration (mg / mL)	Optical Density
200	0.331
500	0.912
1000	1.627
1500	2.42
2000	3

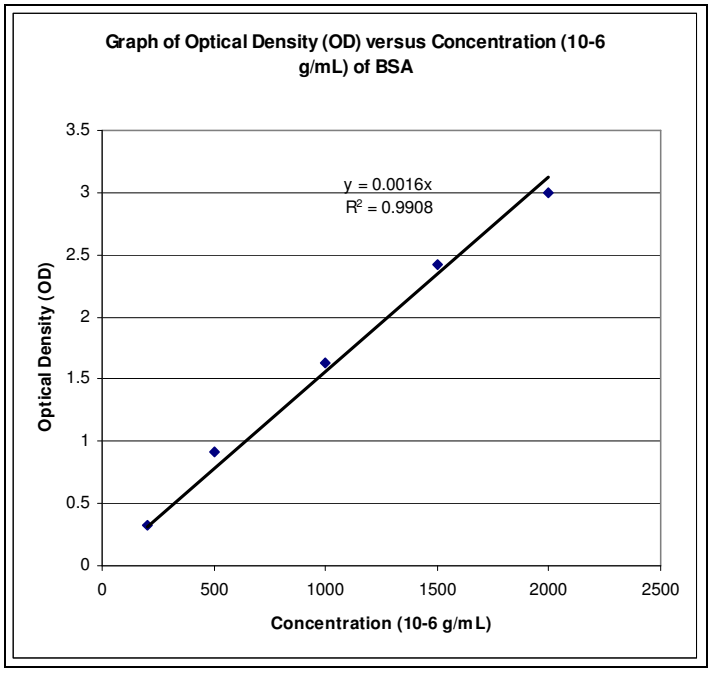

Figure B1 Calibration Curve of BSA protein standard

APPENDIX C

Water Flux Determination

Table C1: Experimental Data of Water Flux Determination

Trial	TMP, (psi)	Temperature (°C)	Volume of Permeate (mL/min)	Flux (LMH)	Flux (LMH/psig at 20 °C)	Membrane Performance Reduction (%)
1	5	23	220	120	23.832	
	10	-	-	-	41.706	**Initial value*
	15	23	550	300	59.58	
2	5	26	230	125.4545		
	10	27	430	234.5455	19.95981818	0.52142
	15	27	615	335.4545		
3	5	22	168	91.63636		
	10	22	330	180	17.19	0.58783
	15	22.5	485	264.5455		
4	5	25	172	93.81818		
	10	24	328	178.9091	16.29861818	0.6092
	15	25	536	292.3636		
5	5	27	168	91.63636		
	10	27	380	207.2727	17.63890909	0.57707
	15	27	640	349.0909		
6	5	24	250	136.3636		
	10	23	420	229.0909	21.37418182	0.4875
	15	23	670	365.4545		
7	5	24	220	120		
	10	24	415	226.3636	20.62172727	0.50555
	15	24	590	321.8182		
8	5	25	150	81.81818		
	10	25	320	174.5455	15.53454545	0.62752
	15	25	400	218.1818		

Table C.2: Viscosity Correction Factor (Kvick Lab Self-Contained Unit Manual)

Temperature in °C when permeate flow was measured	Viscosity correction factor	Temperature in °C when permeate flow was measured	Viscosity correction factor
4	1.567	25	0.890
5	1.519	26	0.871
6	1.472	27	0.851
7	1.428	28	0.833
8	1.386	29	0.815
9	1.346	30	0.798
10	1.307	31	0.781
11	1.271	32	0.765
12	1.235	33	0.749
13	1.202	34	0.734
14	1.169	35	0.719
15	1.139	36	0.705
16	1.109	37	0.692
17	1.081	38	0.678
18	1.053	39	0.665
19	1.027	40	0.653
20	1.000	41	0.641
21	0.978	42	0.629
22	0.955	43	0.618
23	0.933	44	0.607
24	0.911	45	0.592

APPENDIX D

Experimental Data for Transmembrane Pressure (TMP) of 5 psi

Table D1: Experimental Data for Transmembrane Pressure (TMP) of 5 psi

Time (s)	Volume (mL/min)		Flux (LMH)		Optical Density		Concentration (μg/mL)	
	P	***R***	***P***	***R***	***P***	***R***	***P***	***R***
60	36	215	19.6364	117.273	0.391	1.329	1221.88	20765.6
120	34	210	18.5455	114.545	0.12	1.42	375	22187.5
180	34	210	18.5455	114.545	0.375	1.394	1171.88	21781.3
240	32	210	17.4545	114.545	0.07	1.426	218.75	22281.3
300	32	210	17.4545	114.545	0.31	1.42	968.75	22187.5
360	32	210	17.4545	114.545	0.311	1.448	971.875	22625
420	34	210	18.5455	114.545	0.042	1.408	131.25	22000

P = Permeate R = Retentate

Table D2: Dilution Factor for Spectrophotometry

Time (s)	DF	
	Permeate	***Retentate***
60	1:5	1:25
120	1:5	1:25
180	1:5	1:25
240	1:5	1:25
300	1:5	1:25
360	1:5	1:25

APPENDIX E

Experimental Data for Transmembrane Pressure (TMP) of 10 psi

Table E1: Experimental Data for Transmembrane Pressure (TMP) of 10 psi

Time (s)	**Volume (mL/min)**		**Flux (LMH)**		**Optical Density**		**Concentration (μg/mL)**	
	P	***R***	***P***	***R***	***P***	***R***	***P***	***R***
60	44	380	24	207.272	0.268	0.4	837.5	5000
120	42	365	22.9090	199.090	0.393	1.9	1228.13	23750
180	42	360	22.9090	196.363	0.414	0.56	1293.75	7000
240	42	368	22.9090	200.727	0.489	0.505	1528.13	6312.5
300	42	368	22.9090	200.727	0.436	0.491	1362.5	6137.5
360	42	365	22.9090	199.090	0.776	1.302	2425	16275
420	42	375	22.9090	204.545	0.404	1.638	1262.5	20475

P = Permeate R = Retentate

Table E2: Dilution Factor for Spectrophotometry

Time (s)	**DF**	
	Permeate	***Retentate***
60	1:5	1:20
120	1:5	1:20
180	1:5	1:20
240	1:5	1:20
300	1:5	1:20
360	1:5	1:20

APPENDIX F

Experimental Data for Transmembrane Pressure (TMP) of 15 psi

Table F1: Experimental Data for Transmembrane Pressure (TMP) of 15 psi

Time (s)	Volume (mL/min)		Flux (LMH)		Optical Density		Concentration (μg/mL)	
	P	***R***	***P***	***R***	***P***	***R***	***P***	***R***
60	44	290	24	158.182	1.136	1.851	3550	23137.5
120	45	295	24.5455	160.909	0.946	1.833	2956.25	22912.5
180	45	295	24.5455	160.909	1.339	1.755	4184.38	21937.5
240	46	283	25.0909	154.364	1.498	1.94	4681.25	24250
300	44	278	24	151.636	1.635	1.886	5109.38	23575
360	45	278	24.5455	151.636	1.432	1.848	4475	23100
420	45	285	24.5455	155.455	1.55	2.036	4843.75	25450

P = Permeate R = Retentate

Table F2: Dilution Factor for Spectrophotometry

Time (s)	DF	
	Permeate	***Retentate***
60	1:5	1:20
120	1:5	1:20
180	1:5	1:20
240	1:5	1:20
300	1:5	1:20
360	1:5	1:20

APPENDIX G

Experimental Data for Transmembrane Pressure (TMP) of 20 psi

Table G1: Experimental Data for Transmembrane Pressure (TMP) of 20 psi

Time (s)	**Volume (mL/min)**		**Flux (LMH)**		**Optical Density**		**Concentration (μg/mL)**	
	P	***R***	***P***	***R***	***P***	***R***	***P***	***R***
60	40	410	21.8182	223.636	0.615	2.046	1921.88	25575
120	44	395	24	215.455	0.39	1.827	1218.75	22837.5
180	50	390	27.2727	212.727	0.552	1.863	1725	23287.5
240	51	390	27.8182	212.727	0.416	1.936	1300	24200
300	51	390	27.8182	212.727	0.41	1.936	1281.25	24200
360	51	385	27.8182	210	0.46	1.943	1437.5	24287.5
420	51	385	27.8182	210	0.536	2.092	1675	26150

P = Permeate R = Retentate

Table G2: Dilution Factor for Spectrophotometry

Time (s)	**DF**	
	Permeate	***Retentate***
60	1:5	1:20
120	1:5	1:20
180	1:5	1:20
240	1:5	1:20
300	1:5	1:20
360	1:5	1:20

APPENDIX H

Experimental Data for Transmembrane Pressure (TMP) of 25 psi

Table H1: Experimental Data for Transmembrane Pressure (TMP) of 25 psi

Time (s)	**Volume (mL/min)**		**Flux (LMH)**		**Optical Density**		**Concentration (μg/mL)**	
	P	***R***	***P***	***R***	***P***	***R***	***P***	***R***
60	56	320	30.5455	174.545	0.491	2.284	1534.38	28550
120	56	310	30.5455	169.091	0.987	2.62	3084.38	32750
180	58	300	31.6364	163.636	1.37	1.86	4281.25	23250
240	60	300	32.7273	163.636	1.33	3	4156.25	37500
300	58	295	31.6364	160.909	1.478	3	4618.75	37500
360	60	290	32.7273	158.182	1.697	3	5303.13	37500
420	60	295	32.7273	160.909	1.609	2.824	5028.13	35300

P = Permeate R = Retentate

Table H2: Dilution Factor for Spectrophotometry

Time (s)	**DF**	
	Permeate	***Retentate***
60	1:5	1:20
120	1:5	1:20
180	1:5	1:20
240	1:5	1:20
300	1:5	1:20
360	1:5	1:20

APPENDIX I

Experimental Data for Various Transmembrane Pressure (TMP)

Table I1: Experimental Data for Various Transmembrane Pressure (TMP)

TMP (psi)	Volume (mL/min)		Flux (LMH)		Optical Density		Concentration (μg/mL)	
	P	***R***	***P***	***R***	***P***	***R***	***P***	***R***
5	38	95	20.7273	51.8182	0.259	2.678	809.375	41843.8
10	54	245	29.4545	133.636	0.44	2.041	1375	31890.6
15	46	208	25.0909	113.455	0.443	2.036	1384.38	31812.5
20	52	233	28.3636	127.091	0.457	1.867	2856.25	29171.9
25	72	185	39.2727	100.909	0.571	2.468	8921.88	38562.5
30	230	200	125.455	109.091	1.281	1.582	20015.6	24718.8

P = Permeate R = Retentate

Table I2: Dilution Factor for Spectrophotometry

TMP (psi)	DF	
	Permeate	***Retentate***
5	1:5	1:25
10	1:5	1:25
15	1:5	1:25
20	1:10	1:25
25	1:25	1:25
30	1:25	1:25

APPENDIX J

Experimental Pictures

Figure J1 Samples Preparation: Measurement of Samples into Centrifuge Tubes

Figure J2 Samples Preparation: Differential Centrifugation of Samples

Figure J3 Samples Preparation: Separation of Pellets and Supernatant after Differential Centrifugation Process

Figure J4 Samples Preparation: Filtration of Samples into Conical Flask