A COMPARATIVE STUDY ON PROTEIN SEPARATION USING DIFFERENT TECHNIQUES

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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

MAY 2009

I declare that this thesis entitled "A Comparative Study on Protein Separation Using Different Techniques" 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."

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Special Thanks To My Beloved Mother and Father

And

Supportive Lecturer

Ms. Sureena binti Abdullah

ACKNOWLEDGEMENT

First, I wish to express my sincere appreciation to my thesis supervisor, Ms. Sureena binti Abdullah for her guidance and encouragement throughout this study. I would like to pleasure to all technical staff from Faculty of Chemical & Natural Resources Engineering of Universiti Malaysia Pahang (UMP) for their cooperation and endless efforts.

I also want to thankful to all my colleagues and others who have helped me to complete this thesis. Their views and tips are useful indeed. Last, but not least I thank to my family members for their continuous support while completing this thesis.

ABSTRACT

Concentration of commercial soy milk into protein stream using crossflow filtration unit with ultrafiltration membrane and conventional method was examined. Two membranes of molecular weight cut off (MWCO) of 10 and 50 kilo Dalton (kDa) of regenerated polysulfone material were used to determine the better techniques and efficiency of the process. The performance was determined under various processing conditions that include the operating transmembrane pressure the pore size of the membrane. Partially soymilk was centrifugated at 8000 rpm and 30 minutes in $20^{o}C$ to remove the colloidal materials to prevent the membrane fouling. It is found that the permeate flux for 10kDa membrane are decreased with time. This is due to fouling of the membrane. An increased in transmembrane pressure increased the permeate flux and protein weight. The 10kDa membrane shows that the ability to adsorb more amount protein instead of 50kDa membrane. The weight of protein being low at lower transmembrane pressure (less than 10psig) and high at higher transmembrane pressures (greater than 15 psig). The amount of protein recovered at greater transmembrane pressure is more than 5 grams. The method of isolation (conventional method) were adjusted the pH of soy milk (dilute with distilled water) to 4.6 at $20^{o}C$. Used this method gives a weight of protein in not more than 5 grams. It is found that the best membrane to retain highest weight of protein is 10kDa.

ABSTRAK

Kepekatan susu soya komersil telah dikaji dengan menggunakan alat penurasan aliran silang melalui kaedah membran ultraturasan dan konvensional. Membran yang dihasilkan daripada gentian asimetrik terdiri daripada 10 dan 50 kilo Dalton (kDa) telah digunakan bagi menentukan membran yang akan memberi hasil turasan yang terbaik. Penurasan yang terbaik telah dikaji di bawah pelbagai keadaan dengan mengubah tekanan membran dan saiz liang membran. Peningkatan tekanan dalam membrane akan meningkatkan fluks dan jumlah protin. Membran 10kDa mempunyai keupayaan untuk menjerap lebih banyak protin berbanding membran 50kDa pada tekanan rendah (kurang daripada 10psia) dan tekanan tinggi (lebih daripada 15psia). Kadar protin yang terpisah pada tekanan yang tinggi adalah melebihi 5 gram. Salah satu kaedah lain untuk pengasingan protin ialah dengan menggunakan kaedah konvensional. Kaedah ini ialah dengan mengubah pH susu soya kepada 4 pada suhu 20^{o}C. Melalui kaedah ini, jumlah protin yang terpisah adalah tidak melebihi 5 gram. Ini menunjukkan, membrane bersaiz 10kDa dapat memisahkan protin pada kadar yang paling tinggi jika dibandingkan dengan kaedah lain.

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C_p	-	Concentration of permeate
C_R	-	Concentration of retentate
C_i	-	Concentration of protein on cake surface
C_b	-	Concentration of protein in bulk suspension
C_P	-	Concentration of protein in filtrate
h	-	Hour
J	-	Permeate flux
L	-	Liter
m	-	Meter
P_{in}	-	Feed pressure
P_{out}	-	Retentate pressure
P_p	-	Permeate pressure
ΔP	-	Filtration pressure
R_i	-	Protein recovery or retentate recovery
t	-	Time
T	-	Temperature
TMP	-	Transmembrane pressure
U	-	Velocity
V	-	Volume

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Soy protein refers to the protein that is found in soybeans. It is often used to replace animal proteins in an individual's diet. Soybean proteins are important filler in processed meat products including nutritional beverages and are also used extensively in infant formulas. Normally, soy protein is used in many foods as functional and nutritional ingredients. The demand for the food grade proteins were resulted by the complete food formulation from refined ingredients in the present (Chove *et al*., 2006). Regarding to U.S Food and Drug Administration, FDA (1999), soy protein have several physiological functions such as cholesterol-lowering and body-fat reducing effect. It also states that, consuming a diet rich in soy protein has a number of health benefits that are unique to women's needs. The importance of soy milk and soy related products can therefore be summarized by Akoum (2006) 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.

A diet rich in soy protein may alleviate certain symptoms associated with menopause, help reduce the risk of breast cancer, promote heart health and maintain bone health. According to FDA's claims, products that contain high in soy protein may reduce heart disease. They are recommended that consuming twenty five (25) grams of soy protein per day as part of a diet low in saturated fat and cholesterol forheart health. In addition, research shows that Asian populations who regularly consume soy foods have a lower occurrence of heart disease as compared to populations that consume the typical "Western" diet whereby anti-cancer and other health benefits of soy bean products, which has spurred research into better ways to use soy extracts as food ingredients (Chove *et al.*, 2006). Such properties also made soy protein-based foods to become one of the fastest growing categories in the food industry, resulting in the demand for production of soy protein ingredients with improved processing characteristics (Malhotra and Coupland, 2004; Tsumura *et al.*, 2005).

Soy protein production can be improved by using applications of membrane processes. Membrane processes are widely used in the dairy industry, not only for total protein concentration by ultrafiltration for cheese manufacturing or milk standardization and for whey treatment, but also in fractionation of milk proteins and bacteria removal by microfiltration and ultrafiltration. Ultrafiltration of soy milk has been investigated for the concentration of proteins in order to obtain valuable vegetable protein ingredients (Kumar *et al*., 2003).

Selecting an appropriate operating condition, membrane, and element is important to improve the quality of refined soy protein and reduce the production cost. Membrane separation is a technique that permits concentration and separation without the use of heat but for conventional processing methods of soy milk and its products involve heating. It is well known that thermal treatments induce dissociation, denaturation and aggregation of soy protein (Zhang *et al*., 2004).

Cross flow filtration, CFF (microfiltration, MF and ultrafiltration, UF) can be used to fractionate this milk protein (Punidadas and Rizvi, 1998). In CFF, as liquid products flow parallel to the surface of the membrane, the permeate passes 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). The purpose of this work is to make the comparison by using different techniques on protein separation. In this study the effects of cross flow velocity, transmembrane pressure and permeation and soy protein quality were determined.

1.2 Problem Statement

These techniques of membrane technologies offer significant advantages over conventional soy processing methods. Membrane technology has been generally operated at normal temperatures where it requires only simple equipment and small volumes and it has low energy consumption and convenient maintenance instead of conventional method. These processes have the advantages of increased yield.

1.3 Research Objectives

The purpose of this research is to compare the best technique to improve the concentration of the proteins by study the effect of pressure and permeation on protein separation using pasteurized soymilk.

1.4 Research Scope

The scopes for this study are:

- To determine the use of membrane systems for the separation of soy proteins in term of effect on transmembrane pressure in range (5 to 25 psig). Normally, high pressures will denatured the proteins and this process is considered to be energy-efficient and safe compared to the other conventional method.

- Performing the determination of protein concentration, permeate flux, and membrane fouling, all of which can have an effect on the overall performance of these membrane systems.

- The ability to compare the best method of getting more quantity of proteins pasteurized soy milk using different types of membrane and normal isolation.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Soy Protein

Soy is a low cost source of protein that has been consumed in Asian nations for many centuries (Sipos, 2002). This food contains fiber, minerals, and isoflavones (a type of flavonoid), all beneficial nutrients that may contribute to a reduction in chronic disease risk (FDA, 1999). Regular intake of this food is thought to be partially responsible for the lower rates of heart disease, stroke, and cancer observed in Eastern populations (FDA, 1999). Due to recent concerns that have been raised about the use of hormone replacement therapy, many researchers are looking to soy as a possible natural alternative to prevent some of the symptoms associated with menopause (Sipos, 2002). There are many soy products such as soy beans, tofu and soy milk out on the market; however, most of these have undergone such high levels of processing, that much of the nutritional benefit is lost (Sipos, 2002).

Soy protein is a main stay of Asian diets, which typically are either vegetarian or contain small amounts of animal-protein foods. In fact soy provides up to sixty percent of the total dietary protein in some Southern Asian countries (Mary, 2000). Soy protein is used in a variety of foods such as salad dressings, soups, imitation meats, beverage powders, cheeses, non-dairy creamer, frozendesserts, whipped topping, infant formulas, breads, breakfast cereals, pastas and pet foods.

Specific applications include adhesives, asphalts, and resins, cleaning materials, cosmetics, inks, pleather, paints, paper coatings, pesticides/fungicides, plastics, polyesters and textile fibres.

2.1.1 Soy Protein Concentrate

Soy protein concentrate is about seventy percent soy protein and is basically soybean without the water soluble carbohydrates. It is made by removing part of the carbohydrates (sugars) dehulled and defatted soy beans. Soy protein concentrate retains most of the fiber of the original soybean. Soy protein concentrate is widely used as functional or nutritional ingredient in a wide variety of food products, mainly in baked foods, breakfast cereals and in some meat products. Soy protein concentrate is used in meat and poultry products to increase water and fat retention, and to improved nutritional values. Soy protein concentrates are available in different forms; granules, flour and spray dried. Because they are very digestible, they are well-suited for children, pregnant and lactating women and the elderly.

2.1.2 Soy Protein Isolate

Soy protein isolate is a highly refined or purified form of soy protein with a minimum protein content of ninety percent on a moisture-free basis (Josepth, 2003). It is made from defatted soy flour which has had most of the non-protein components, fats and carbohydrates removed. Soy isolates are mainly used to improve the texture of meat products, but are also used to increase protein content and enhance flavor.

2.1.3 Nutritional Value of Soy Protein

Regarding to Sipos (2002), under conditions of normal dietary intake, properly processed soy protein ingredients are of good protein value for humans and the nutritional adequacy of soy protein products has been clearly demonstrated in infant formulas, where protein requirements are most critical. Soy protein contains all the essential amino acids required for human nutrition (growth, maintenance, and stress) (FDA, 1999). The amino acid composition of soy protein resembles, with the exception of the sulfur-containing amino acids (e.g., methionine), the amino acid patterns of high quality animal protein sources (FDA, 1999).

There is suggestion that soy protein may offer positive health benefits of significant proportions (FDA, 1999). Animal studies shows that animal protein (usually casein) is more cholesterolemic and atherogenic than vegetable protein (most frequently mentioned soy protein). In man, too, vegetable protein appears to be less cholesterolemic than animal protein (Sipos, 2002). The difference persists even in the face of high levels of saturated fat consumption.

2.1.4 Functional of Soy Protein

Functional properties are not only important in determining the quality of the final product, but also in facilitating processing, e.g., improved mach inability of cookie dough or slicing of processed meats (Sipos, 2002). These properties were attributed to the protein; however, other components in certain products may also contribute to functionality. For example, polysaccharides in soy flour and concentrate will absorb more water than an equivalent amount of protein (Sipos, 2002).

Product characteristics of soy protein products can be various by using different processing treatments. These treatments can involve the use of enzymes, solvents, heat, fractionation, and pH adjustment, or the combination of these treatments (Sipos, 2002).

At present, soy proteins are more flexible than many other food proteins in various worldwide nutritional programs. While soy protein products acceptance has grown because of such functional properties, abundance and low cost (Josepth, 2001).

2.1.5 The Important of Protein

Protein is a very important part of all living tissues. Regarding to Mary 2001, the protein primary functions are to build new tissue during period of growth and heating. It is also repair and maintain the tissue of body. Food proteins are often classified as complete or incomplete protein. Complete food protein contain all of essential amino acids in the necessary amounts to carry out protein's functions in the body. Incomplete proteins are lack of sufficient amounts of one or more of the essential amino acids (Mary, 2000).

2.2 Filtration

Filtration is an operation that has found in the processing of biotechnology products (Harrison *et al.,* 2003). In general, filtration is used to separate particulate or solute components in a fluid suspension or solution according to their size by flowing under a pressure diffential through a porous medium (Harrison *et al.,* 2003). There are two categories of filtration, which is conventional or dead-end filtration, where the fluid flows perpendicular to the medium. It will results in a cake of solid depositing on the filter medium. While for the crossflow filtration, the fluid flows parallel to the medium to minimize buildup of solids on the medium. In this research, crossflow filtration (which is called tangential flow filtration), is used to separate the soy protein from the soybean solution.

2.2.1 Crossflow Filtration

In crossflow filtration, the fluid flows parallel to the medium resulting in constant permeate flux at steady state (Roger *et. al.*, 2003). It has been used in variety of application including the concentration of protein solutions, the removal of salts in protein solutions and the removal of viruses from protein solution. Crossflow filtration can be divided into two categories, that is ultrafilration membranes (UF) are generally used when dissolve species such as proteins are being filtered and also for microfiltration membrane (MF) where it is used for the separation of casein and whey proteins from milk. When the dissolve species such as proteins are being filtered, ultrafiltration membrane is generally used (Roger *et. al.*, 2003). The ultrafiltration membrane is selected so that the species of interest will not pass through the membrane. The theory for the crossflow filtration of dissolve species has been found to hold only for very small suspended particles up to approximately 1 µm in size (Belfort *et.al.*, 1994). For crossflow filtration with microporous filtration membranes, the cake layer initially grows with time and reducing the permeate flux (Roger *et. al.*, 2003).

2.2.1.1 Ultrafiltration

Ultrafiltration (UF) is a pressure driven, separation process in which membranes having pore sizes ranging from 10-1000 Å. This membrane are used for the concentration, dialfiltration, clarification and fractionation of macromolecules (e.g. proteins, nucleic acids, and synthetic polymers) (Kulkarni *et al.,* 1992). Membrane based separation process are generally rely on the type of material being processed. A membrane usually used to retain a smaller molecule while allowing a larger molecule to pass trough (Higushi *et al.,* 1991). Separation occurs based on molecular size and chemical interactions between the membrane and fluid components that are in contact with the membrane (Wheelwright, 1991). UF is an effective technique for concentrating and separating dissolves molecules in different sizes and typical operating pressures range from 30-150 psi (Wheelwright, 1991). The membrane should be strong enough to withstand this applied pressure. It should also be able to withstand shear force resulting from material flow, as well as vibrations cause by other system components such as pump and also be mechanically durable to withstand reuse, repeated dismantling of the membrane module and cleaning procedures (Raja Gosh, 2003).

2.2.1.2 Microfiltration

Microfiltration, (MF) is a pressure-driven membrane process for the separation of fine particles, microorganisms and emulsion droplets. The membranes used have a microporous structure which separates fine particles with a size in the range of 0.02–20 microns. Therefore, MF is placed between ultrafiltration and coarse filtration, which is not a membrane operation. In CMF, the fluid to be filtered flows parallel to the membrane surface and permeates through the membrane due to a pressure difference (Ripperger *et al*., 2002). The crossflow reduces the formation of a filter cake and keeps it at a low level. So it is possible to get a quasi-steady filtrate flow for a long time (Ripperger *et al.*, 2002).

In MF the membrane has a microporous structure and separates particles according to the size of pores, from a liquid or a gas phase. The separation is based on the sieve effect. Therefore, the separation effect is mostly limited to the outer surface of the membrane (Ripperger *et al*., 2002). The microporous structure of the membrane should have a narrow pore size distribution in order to ensure the quantitative retention of particles of a given size and type. Another important feature