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EFFECT OF DIAFILTRATION ON PREPARATION OF FERMENTED MUNG BEANS CONCENTRATE AS PROBIOTIC SAVORY FLAVOR THROUGH ULTRAFILTRATION MEMBRANE

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Abstract

Diafiltration by means of the ultrafiltration system of probiotic fermented Mung beans (*Phaseolus radiatus* L.) concentrate has been performed to reduce or eliminate salts and smaller impurities than the nominal cut-off of the membrane of 20,000 nominal weight cut-off (NWCO). These processes have been conducted as an attempt in order to get a probiotic product with organoleptic acceptability, composition, and the optimal total lactic acid bacteria (LAB) counts because the presence of salts will affect on the viability of LAB and the cell lysis of LAB and limit its utility in food products. Concentrate of probiotic mung beans was prepared through fermentation of LAB using inoculum of LAB consisting of *Lactobacillus bulgaricus* and *Streptococcus thermophylus* (1 : 1) on fermented mung beans extract inoculated by inoculum of *Rhizopus*–C_1 in rice substrates at salt condition. Ultrafiltration and diafiltration modes have been carried out at flow rate of 8.77 Liter/minute, room temperature and the pressure of 5 bar (0 to 79.7 minutes) and 7 bar (0-154.5 minutes) with the ratio of the volume of pure water to the volume of initial feed (number of diavolume, N_d) of 0, 0.25, 0.5, 0.75, 1.0 and 1.25, respectively. The experiment results based on total LAB counts as a probiotic product show that a high N_d can reduce the salt content but increase the total LAB counts. N_d of 1.0 results reduce the salt content which is equal to retentate, permeate, and the optimal total LAB counts. Ultrafiltration and diafiltration modes at the pressure of 7 bar and N_d of 1.0 give a retentate with total solid of 6.1355%, salt of 1.3515% and remove 86.15% of the salt from probiotic fermented mung beans concentrate and total LAB counts of 10.73 log cycles. Meanwhile, the permeate obtained at this condition results in flux value of 10.83 Liter/m^2.hour with contents of total solid of 6.8199%, salt of 1.325% and total LAB counts of 5.49 log cycles.

Keywords: diafiltration, fermented mung beans extract, LAB, probiotic, salt removal, ultrafiltration membrane

1. Introduction

Beans, such as mung beans (*Phaseolus radiatus* L.) and kidney beans (*Phaseolus vulgaris* L.), have been a staple part or diets for thousands of years and and are widely consumed in East Asian countries (China, Korea, Japan, and Taiwan) and South-East Asian countries, such as Malaysia, Singapore, Thailand, Vietnam, and Indonesia today. They have high complex carbohydrate, protein, and fiber, but are extremely low in fat. Mung beans or kidney beans have been the primary source of protein for utility as functional ingredients in food systems. The functional properties of protein in mung beans or kidney beans commodity play a larger role than nutritional considerations in determining their acceptability as valuable ingredients in prepared foods. With the growing interest in fitness and health, and in reducing the consumption of animal protein products for health and economic reasons, there are signs that vegetable protein will constitute a much higher proportion of the human diet in the future [1].

One of the beans manufacturing is an application of fermentation process of salt on mung beans using inoculum of *Rhizopus*–C_1 then the inoculated by Lactic Acid Bacteria (LAB) using *Lactobacillus bulgaricus* and *Streptococcus thermophillus* as probiotic ingredients. The fermentation of mung beans, as in the case of fermented vegetables, occurs mainly in the brine condition. The transformation of the salt into a culture medium, at the expense of the components contained in the mung beans, is gradual; its rate depends on many factors, such as the variety of mung beans used, mung beans/salt/inoculum proportion, fermentation temperature and time, kind and concentration of inoculum, and so on. This product of salt fermentation process is crude fermented mung beans that require further processes consisting of extraction, homogenization, first and

second filtration and microfiltration with the high salt content. The high salt content of crude fermented mung beans product limits its utility in food products [2]. In order to reduce or eliminate high salt content of crude fermented mung bean product, ultrafiltration can provide further product improvement to add, such as taste, nutritional quality, and organoleptic acceptability, through the removal of salts (and undesirable impurities) from the extract of fermented mung beans by operating in the diafiltration mode [3].

Ultrafiltration is primarily a pressure driven membrane separation process based on sieving mechanism in which suspended particles are separated from solution or suspension based on size; however, other factors, such as wetting, solubility, and ionic interactions can affect performance, such as flux and selectivity. Ultrafiltration is used to separate material in the 1 to 100 nm in size range or particles with molecular weight from 1,000 to 1,000,000 Dalton [4-6]. Because of many unique properties of membrane technology, such as gentle thermal effect (consume less energy), no phase change, no chemical addition, minimum disposal problems, the increase of product recovery, the raise of product purity, operation at low, ambient and high temperature, and simple operation ultrafiltration, membrane processes mainly provide a better option over the conventional separation methods in agro-food processes (sugar refining, cheese processing), pharma-ceutical and biotechnological (enzyme, fermentation broth) fields. However, important limitation to the membrane process is that the solutes can not be taken to dryness and limited by higher solids content. Because the osmotic pressure of the retained macromolecules is very low, mass transfer rate obtained with concentrated macromolecules is low, and viscosity of suspension is high, making the pumping of the retentate difficult. Skim milk has been thought to be economically concentrated to 38% solids content [3,7,8]. Study on separation of vegetable protein by means of membrane filtration systems were first conducted in the early - to mid 1970s. The system produced a full fat soy protein concentrate by ultrafiltration using diafiltration mode. This diafiltration mode was found in removing the oligosaccharides from the full fat soy bean extract [9].

Diafiltration is the addition of pure water or fresh buffer to the feed tank at the same rate as permeate withdrawn, thereby maintaining constant feed or retentate volume. During diafiltration, pure water or fresh buffer is introduced into the feed tank while permeate is removed from the unit operation. In processes where the product in the retentate, diafiltration washes components out of the product pool into the permeate, thereby exchanging fresh buffer and reducing the concentration of undesirable impurities. Diafiltration refers to the processes by which ultrafiltration can rapidly and efficiently remove salts and/or lower molecular weight components from larger macromolecules [7,10].

The objective of this experiment is to find out the performance of ultrafiltration and diafiltration mode for the removal of salt and the increase of total LAB counts contained in probiotic fermented mung beans in order to improve their nutritional quality and organoleptic acceptability in food system.

2. Methods

Substances and Equipment. Substances used in this experiment were extract of fermented mung beans using starter of *Rhizopus*–C_1, inoculum of LAB, namely the probiotic cultures containing mixed strain of *L. bulgaricus* and *S. thermophillus* (1 : 1) supplied from Research Centre for Chemistry - Indonesian Institute of Sciences, skim milk as source of lactose on LAB, sucrose of 12% and chemicals for analyses composition of nutrition facts and microbiology aspect. All chemicals were reagent grade obtained commercially. The commercial polysulphone membrane (GR-61-PP) with a nominal molecular weight cut-off of 20,000 Dalton was purchased from DSS, Denmark. This membrane is in a flat-sheet form and has an effective filtration area of 360 cm^2. The operating pressure is 1 - 13 bar and operating temperature 0-75 ^oC. The allowed pH range is from 1 to 13.

The equipment utilized in this work was fermentation system of laboratory scale, trays, autoclave, homogenizer, sieve screen of 140 mesh, plate & frame type cross-flow membrane filtration module unit (LabStak® M20-0.72-PSO, DSS, Denmark) [11], and ATAGO salinity meter (Japan).

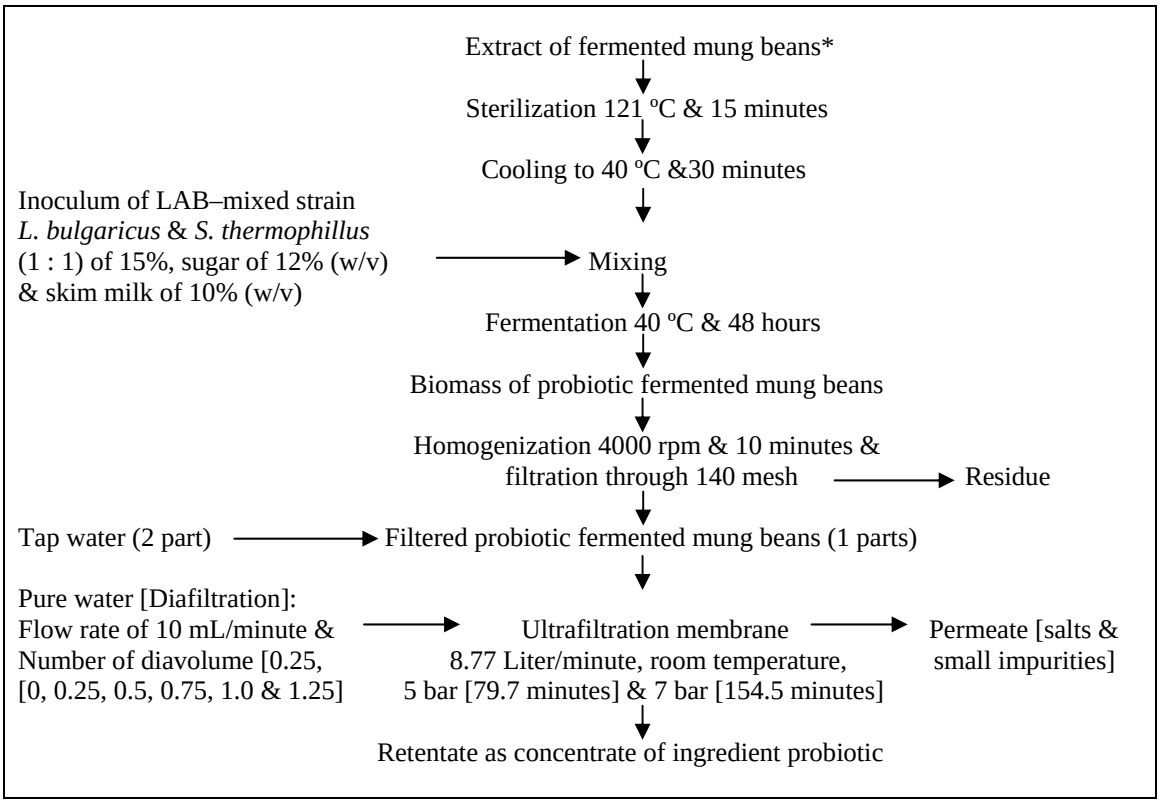
Experimental Design. This experiment was conducted with at pump motor frequency of 25 Hz (flow rate of 8.77 Liter/minute), room temperature, and a trans membrane pressure of 5 and 7 bar and number of diavolume (N_d) 0, 0.25, 0.5, 0.75, 1.0, and 1.25, respectively on the results of concentration of biomass of fermented mung beans using *Rhizopus* sp-C1 inoculated with inoculum of LAB as the probiotic cultures containing mixed strain of *L. bulgaricus* and *S. thermophillus* (1 : 1). Number of diavolume (N_d) is ratio of volume of fresh water added to initial volume of solution in feed tank.

Analyses were performed on the composition of extract of probiotic fermented mung beans, such as total solids, total protein, dissolved protein, N-amino, reduction sugar, salt, total acid, total LAB counts and pH. Investigation of ultrafiltration membrane performance was permeate flux value and chemical composition, such as total solids, salt, and total LAB counts in

retentate and permeate. Total solid, salt and total LAB counts were determined according to standard AOAC methods. Total solid was determined by the gravimetry method. Salt content was measured using the ATAGO salinity meter. Total LAB counts were counted on MRS agar (OXOID) used pour plate (plate count method). Plates were incubated at 30 ^{o}C for 48 hours.

Preparation, concentration, and diafiltration processes of biomass of probiotic fermented mung beans. Substances used in this experiment were extract of fermented mung beans using starter of *Rhizopus*–C_1, and this product was then extracted with an additional tap water 80 ºC at fermented mung beans and total water ratios of 1 : 8 (w/w). Further, this product was sterilized using autoclave at 121 ºC for 15 minutes, cooled to 40 ºC for 30 minutes, inoculated with LAB inoculum–mixed strain (*L. bulgaricus* & *S. thermophyllus* (1 : 1) of 15%, sucrose of 12% (w/v) and skim milk as source of lactose on LAB of 10% (w/v), mixed, fermented at 40 ºC for 48 hours and produced biomass of probiotic fermented mung beans. This biomass of probiotic was homogenized at 4000 rpm for 10 minutes and filtered through sieve screen of 140 mesh so that it resulted in filtered probiotic fermented mung beans and residue. To make suspension of probiotic product, an additional of tap water to filtered probiotic fermented mung beans was carried out at ratio of 2 and 1. The results of these above processes are probitic fermented mung beans products as feed during concentration using ultrafiltration membrane and diafiltration mode.

Biomass of probiotic fermented mung beans suspension placed in feed tank of 9 Liter was pumped tangentially over the ultrafiltration membrane by using a positive displacement pump rannie 25.38 with a pump motor frequency of 25 Hz (flow rate of ~ 8.77 Liter/minute), pressure of 5 and 7 bar, respectively. The ultrafiltration module was operated in the batch non-concentration mode keeping the initial feed volume of probiotic fermented mung beans suspension at 2 Liters. Trans membrane pressure was computed as the mean value of the feed and retentate membrane module pressures. The flow rate in the retentate stream was measured and recorded. Flow rate and pressure in the retentate stream were independently controlled by means of the pump motor control and regulation and a neddle valve placedat the retentate side of the membrane module. All the experiments were carried out with retentate recycling. Permeate was recirculated in experiments in which concentration was kept constant and removed from the module. The permeate was recirculated for 15



* Result from salt fermentation of mung beans using starter of *Rhizopus*-C_1, extraction by adding fresh water at crude broth & total water ratio of 1 : 8.

Figure 1. Flow Diagram of Preparation of Fermented Mung Beans Concentrate as Ingredient Probiotic Inoculated Using Inoculum of LAB and Concentration Through Ultrafiltration Membrane and Diafiltration Mode is Shown in Figure 1

minutes before the concentration process started. In diafiltration approach, a batch of the probiotic fermented mung beans suspension to be desalted is maintained at constant volume by adding pure water (dialysate) at the same rate as permeate is removed. In this way, the macromolecule, especially total solids, LAB, protein retained by the membrane remains at initial concentration, while the salt and small impurity concentration decreases continuously. Volumetric permeate flux was measured continuously until steady state was reached. Retentate and permeate were regularly sampled for analyses of their total solid, salt, and total LAB counts. The length of experimental runs was 0–79.7 minutes for the pressure of 5 bar and 0–154.5 minutes for the pressure of 7 bar. After the concentration process was finished, membranes in module were cleaned in place by pumping NaOH solution of 0.1 M at 3.5 L/minute and 60 ºC for 30 minutes and rinsed by reverse osmosis water. Flow diagram of preparation of fermented mung beans concentrate as ingredient probiotic inoculated using inoculum of LAB and concentration through ultrafiltration membrane and diafiltration mode is shown in Figure 1.

3. Results and Discussion

Characteristics of the extract of fermented mung beans as a probiotic ingredient. The extract of fermented mung beans as a probiotic ingredient obtained through fermentation of LAB using inoculum from mixed strain of *L. bulgaricus* dan *S. thermophillus* has some characteristics summarized in Table 1. Based on the composition of fermented mung beans extract, dissolved protein and total LAB counts are the most important components in probiotic products. This case is related with product function for probiotic or prebiotic food ingredients as the source of vegetable protein and probiotic agent for products of vegetable foods. High total LAB counts (6.23 log cycles) are identified that this initial product has been in standard probiotic foods with total LAB counts of $>10^6$ cell/mL [12]. The other objectives of probiotic product are as forticification for food based umami with high content of dissolved protein (0.6 mg/mL) in which all these products indicate high quantities of amino acids and low molecular weight peptides supporting functional properties as the source of savory flavor. Contents of total acids (0.777%) and reduction sugar (50 mg/mL) are the result of specific fermentation of LAB by *L. bulgaricus* and *S. thermophyllus*. High salt content (2.756%) shows the presence of effect of initial substances, such as crude broth produced through process of salt fermentation as basic reaction of this product. Salt fermentation of mung beans using inoculum of *Rhizophus* sp-C1 of 26%, salt of 23% and mung beans of 51% produce fermented mung beans with specific taste as product from converting protein, carbohydrate and fat by activities of protease, amylase, and lipase enzymes, but this product of fermented mung beans contains high salt for products of savory [3].

Table 1. Characteristics of Extract of Fermented Mung Beans as a Probiotic Ingredient as Feed During Concentration Utilizing Ultrafiltration Membrane and Diafiltration Mode

Components	Value
Total solid, %	15.0485
Total protein,% (dry matter)	9.01
Dissolved protein, mg/mL	0.6
N-amino, mg/mL	7.7
Reduction sugar, mg/mL	50
Total acids, %	0.777
Salt, %	2.756
Total LAB counts, log cycle	6.23
pH	3.0

Ultrafiltration and continuous diafiltration of probiotic fermented mung beans extract. The effect of the number of diavolume (N_d) on flux through ultrafiltration membrane and diafiltration mode using probiotic fermented mung beans extract at flow rate of 8.77 Liter/minute, room temperature and pressure of 5 and 7 bar is shown in Figure 2. As demonstrated in Figure 2, for pressure 5 bar, the permeate flux through the membrane matrix drops slowly within the first N_d of 0–0.5, namely 12.63 to 11.8 Liter/m^2.hour, followed by a gradual increase to N_d of 0.75 with flux of 15.01 Liter/m^2.hour and slight decrease toward a limit flux value of approximately 14.84 Liter/m^2.hour at N_d of 1.25. The entire process of ultrafiltration membrane and diafiltration mode using probiotic fermented mung beans extract at pressure 5 bar was completed in 79.7 minutes. The fall in flux value demonstrates that absorption influence probably occurs on a membrane surface or within the secondary layer (concentration polarization). N_d is defined as the ratio of volume of pure water (fresh solvent) added to the volume of initial feed solution to be washed at any time during ultrafiltration and diafiltration. Flux value at 5 bar for N_d of 0, 0.25, 0.5, 0.75, 1.0 and 1.25, is 12.63, 11.97, 11.8, 15.01, 14.6 and 14.84 Liter/m^2.hour, respectively. In the meanwhile for pressure 7 bar, the permeate flux value through the membrane declines rapidly within the first N_d of 0–0.25, namely 24.82 to 12.59 Liter/m^2.hour, followed by a slight decrease toward a limit flux value of approximately of 10.54 Liter/m^2.hour. In this condition, the ultrafiltration is governed by a cake filtration fouling mechanism. This means that the convective permeate flux direct from the bulk solution toward the membrane prevails on the rate of shear-induced back diffusion of the rejected solutes, thus leading to the formation of a cake layer on the membrane surface. The entire process

of ultrafiltration membrane and diafiltration mode using probiotic fermented mung beans extract at pressure 7 bar was completed in 154.5 minutes. Flux value at 7 bar for N_d of 0, 0.25, 0.5, 0.75, 1.0, and 1.25, is 24.82, 12.59, 11.54, 11.12, 10.83 and 10.54 Liter/m^2.hour, respectively. Flux decay over N_d is characterized by two regions. After the first sharp decline, down to a level where a hydrodynamic state of equilibrium for the transport of particles to/and away from the membrane surface can be assumed, a further slower decrease (more or less asymptotically) is seen, which might be caused by compaction and alteration of the layer under the forces acting on it. Besides the hydrodynamic parameters, such as trans -membrane pressure and tangential velocity, fouling factors contribute to the flux decline. Fouling is defined as a phenomenon that is ubiquitous in pressure-driven membrane processes, such as ultrafiltration that can result in the loss of performance of a membrane. Fouling of membrane can occur through different mechanisms, such as absorbing irreversible or reversible near or on to the surface of the membrane (solute-solute interactions), absorption of suspended or dissolved substances at the pore openings or with its pores (solute-membrane interaction), plugging of the pores by particles and the build-up of filter cake (concentration polarization effects).

Total solid content in the retentate and permeate as a function of Number of Diavolume (N_d) using probitic fermented mung beans extract as feed through ultrafiltration membrane and diafiltration mode under flow rate of 8.77 Liter/minute, room temperature and pressure of 5 and 7 bar, respectively is shown in Figure 3. As indicated in Figure 3, under conditions of flow rater of 8.77 Liter/minute, room temperature and pressure 5 bar for time with range between 0 to 79.7 minutes, total solid content decreases with the increase of N_d both in retentate and in permeate. The total solid content of product in retentate with N_d of 0, 0.25, 0.5, 0.75, 1.0 and 1.025, is 15.0485, 7.7289, 5.9476, 4.5942, 3.4191 and 1.2466%, respectively. On the other hand, the total solid content of product in permeate with N_d of 0, 0.25, 0.5, 0.75, 1.0 and 1.25, is 10.3398, 9.2709, 4.6829, 4.0625, 4.6663 and 2.0075%, respectively. The decrease of total solid content of products in retentate and permeate at pressure of 5 bar is caused by its presence of remaining water in retentate as the consequence of the addition of water mass to feed tank during diafiltration process. Solid particleshavinga smaller size penetrate the internal structure of the membrane as permeate due to the solute-membrane interaction (solute adsorption within the porous structure of the membrane) and membrane fouling. Solid particles which do not pass through the membrane pores form a cake on the membrane surfaces.

For condition of flow rate of 8.77 Liter/minute, room temperature and pressure of 7 bar for 0 to 154.5 minutes, total solid content decreases with the increase of N_d both in retentate and permeate. The total solid content of products in retentate with N_d of 0, 0.25, 0.5, 0.75, 1.0 and 1.25, is 15.0485, 13.5257, 12.2024, 7.0982, 6.1355, and 2.756%, respectively,while the total solid content of product in permeate with N_d of of 0, 0.25, 0.5, 0.75, 1.0, and 1.25, is 10.3398, 12.6496, 12.7017, 7.8343, 6.8199, and 2.3761%, respectively. The decrease of total solid content of products in retentate and permeate at pressure of 5 bar is caused by its presence of remaining water in retentate as the consequence of the addition of water mass to feed tank during diafiltration process. Solid particles having a smaller size penetrate the internal structure of the membrane as permeate due to the solute-membrane interaction (solute absorption within the porous structure of the membrane) and membrane fouling. Solid particles which do not pass through the membrane pores form a cake on the membrane surfaces.

Retention of salt during continuous diafiltration of products in a flat sheet polysulphone ultrafiltration membrane at flow rate of 8.77 Liter/minute, room temperature, and pressure of 5 and 7 bar is indicated in

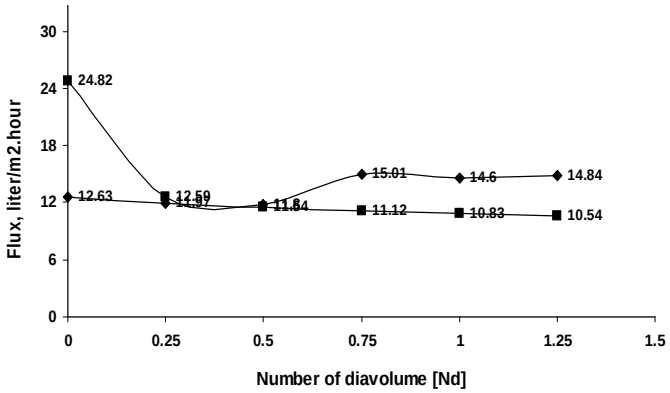


Figure 2. Effect of Number of Diavolume (N_d) on Flux Through Ultrafiltration Membrane and Diafiltration Mode Using Probiotic Fermented Mung Beans Extract at Flow Rate Of 8.77 Liter/Minute, Room Temperature and Pressure of 5 Bar (♦; 0-70.7 Minutes) and 7 Bar (■; 0-154.5 Minutes)

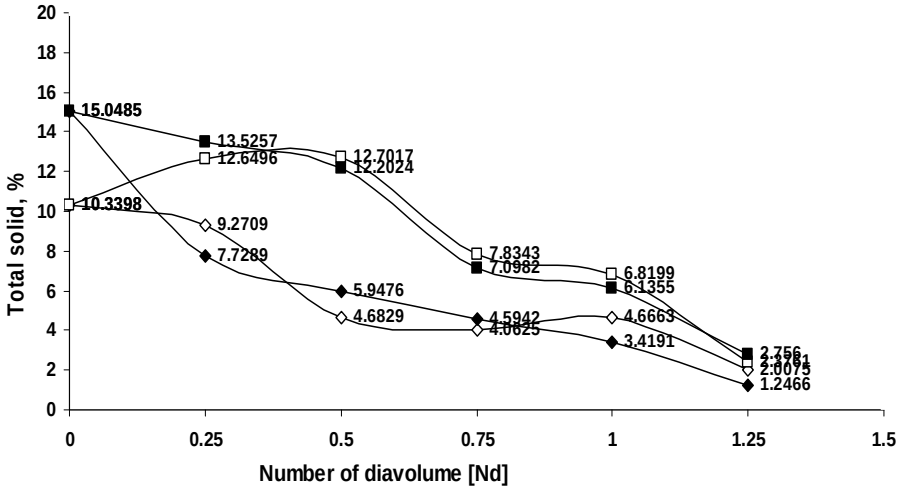


Figure 3. Total Solid in the Retentate & Permeate as a Function of the Number of Diavolume Using Probiotic Fermented Mung Beans Extract Through Ultrafiltration Membrane and Diafiltration Mode Under Flow Rate 8.77 Liter/Minute, Room Temperature and Pressure 5 Bar Permeate (◊), 7 Bar Permeate (□), and 5 Bar Retentate (♦)7 Bar Retentate (■)

As shown in Figure 4, the effectiveness of polysulphone ultrafiltration membrane in course of the diafiltration both at pressure of 5 and 7 bar was then evaluated for the salt removal of products. Diafiltration was performed in the continuous mode in which the permeate flux value was compensated by an equal input of pure water. In this case, salt in the feed/retentate was removed with the permeate flux. In this figure, it can be shown that for N_d of 1.25 at pressure of 5 bar (2500 mL of pure water added to the initial 2000 mL of probiotic fermented mung beans extract) the salt content of product in the retentate was reduced by 86.1% (from 2.756 to 0.383%). The salt content of product in permeate at pressure of 5 bar and N_d of 0, 0.25, 0.5, 0.75, 1,0 and 1.25, is 2.438, 2.2525, 1.59, 1.1925, 0.795 and 0.53%, respectively, whereas the salt content of product in retentate with N_d of 0, 0.25, 0.5, 0.75, 1.0, and 1.25, is 2.756, 2.385, 1.855, 1.272, 0.848 and 0,383%, respectively. Meanwhile for N_d of 1.25 at pressure of 7 bar (2500 mL of pure water added to the initial 2000 mL of probiotic fermented mungs bean extract), the salt content of product in the retentate was reduced by 86.15% (from 3.8263 to 0.53%). The salt content of product in retentate with N_d of 0, 0.25, 0.5, 0.75, 1.0 and 1.25, is 3.8263, 3.604, 3.074, 2.12, 1.3515, and 0.53%, respectively, whereas the salt content of product in permeate with N_d of 0, 0.25, 0.5, 0.75, 1.0 and 1.25, is 4.2882, 3.074, 2.65, 1.908, 1.325 and 0,53%, respectively. In other words, the experiment results of salt in the diafiltration mode both at pressure of 5 and 7 bar are in good agreement. According to the technical result, a diafiltration is able to remove 86.1% of the salt from a product at pressure of 5 bar and 86.15% of the salt from a product at pressure of 7 bar. Because the salt content in retentate both at pressure of 5 and 7 bar decreases, even though the salt content in permeate both at pressure of 5 and 7 bar decreases with more and more high N_d, it is possible to form gel layer at membrane surface. The gel layer can be an actual gel of salt molecules that can be formed without phase separation. The total time including process time of ultrafiltration and time of diafiltration at flow rate of 8.77 Liter/minute, room temperature and pressure of 5 bar is 79.7 minutes, while the total time including process time of ultrafiltration and time of diafiltration at flow rate of 8.77 Liter/minute, room temperature and pressure of 7 bar is 154.5 minutes. In this case, the ultrafiltration process requires the same amount of time by diafiltration. In other words, the ultrafiltration process and diafiltration mode are performed in one step both at pressure 5 and 7 bar.

The change in total LAB counts of product in retentate and permeate during ultrafiltration and diafiltration mode with various N_d at flow rate of 8.77 Liter/minute and room temperature for pressure of 5 bar (0 - 79.7 minutes) and 7 bar (0 - 154.5 minutes) respectively is indicated in Figure 5. As indicated in Figure 5, the total LAB counts of products in retentate at pressure of 5 bar decrease gradually within the N_d from 0 to 1.25. The total LAB counts of products in retentate with N_d of 0, 0.25, 0.5, 0.75, 1.0 and 1.25 are 8.3, 6.46, 6.45, 6.38, 6.28 and 7.94 log cycles, respectively. Meanwhile, the total LAB counts of products in permeate fluctuate with various $N_{d(s)}$. The total LAB counts of products in permeate with N_d 0, 0.25, 0.5, 0.75, 1.0 and 1.25 are 6.43, 0, 6.58, 4, 5.92, and 0 log cycles, respectively. For a pressure of 7 bar, more and more high N_d will gradually rise the total LAB counts of product in retentate. The total LAB counts of product in retentate with N_d 0, 0.25, 0.5, 0.75, 1.0, and 1.25 are 8.3, 8.72, 10.36, 10.72, 10.73, and 10.76 log cycles, respectively, while the total LAB counts of products in permeate

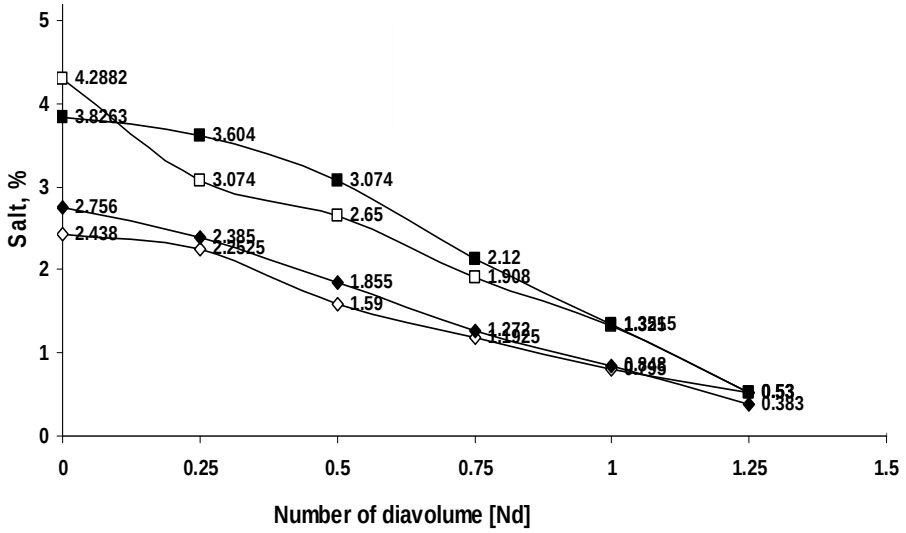


Figure 4. Salt in the Retentate & Permeate as a Function of the Number of Diavolume Using Probiotic Fermented Mung Beans Extract Through Ultrafiltration Membrane and Diafiltration Mode Under Flow Rate 8.77 Liter/Minute, Room Temperature and Pressure 5 Bar Permeate (◊), 7 Bar Permeate (□),5 Bar Retentate (♦), and 7 Bar Retentate (■)

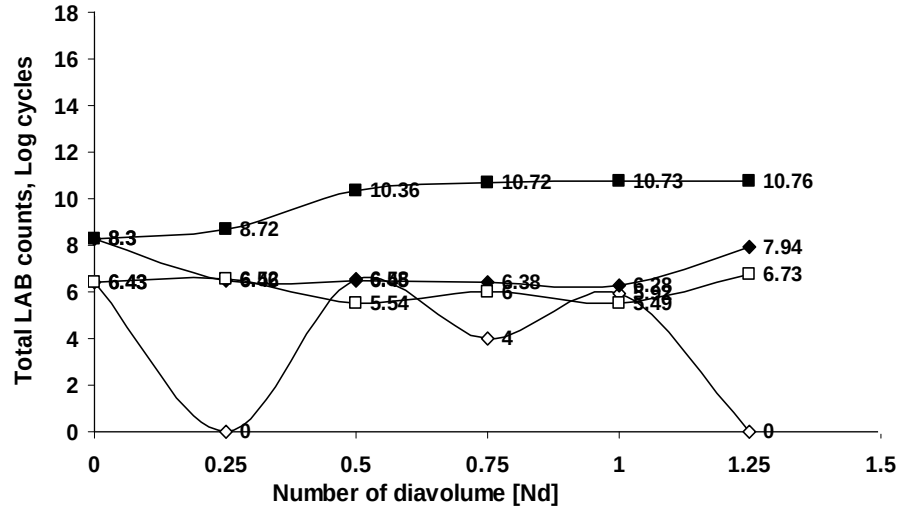


Figure 5. Total LAB Counts in the Retentate & Permeate as a Function of the Number of Diavolume Using Probiotic Fermented Mung Beans Extract Through Ultrafiltration Membrane and Diafiltration Mode Under Flow Rate 8.77 Liter/Minute, Room Temperature and Pressure of 5 Bar Permeate (◊), 7 Bar Permeate (□), 5 Bar Retentate (♦), and 7 Bar Retentate (■)

fluctuate gradually with N_d of 0, 0.25, 0.5, 0.75, 1.0 and 1.25, which is 6.43, 6.52, 5.54, 6, 5.49 and 6.73 log cycles, respectively. However, for these process conditions, not all total LAB counts remain at the membrane surface. Traditionally, membranes are usually rated in respect to the mean pore size. The microorganism cells show some variation in size so that potential leakage of microorganism cells is possible. We recognize that microorganism cells under the fluid forces might change their shape and are able to penetrate to the other side of the membrane matrix (permeate). The penetration process may occur even if the nominal size of microorganism cells under no stress condition is larger than the available pore size. Apart from that, factors which have the potential to cause LAB cells damage are fluid shear and turbulence, damaged by pumping and trans -membrane pressure induced pore deformation and cell lysis in the pores of the membrane.

4. Conclusion

The experiment result shows that the high number of diavolume (N_d) in ultrafiltration and diafiltration mode of probiotic fermented mung beans extract is technically able to reduce the salt content and increase the total

LAB counts. Ultrafiltration and diafiltration mode at flow rate of 8.77 Liter/minute, room temperature and pressure of 7 bar for N_d of 1.25 give a retentate with total solid of 2.75 %, removing 86.15 % of the salt from product, and total LAB counts of 10.76 log cycle and permeate give flux value of 10.83 Liters/m^2.hour. The application of diafiltration mode in probiotic fermented mung beans is still in the beginning. Further experiment is still being conducted to confirm the above result and to arrive at a deeper understanding of the ultrafiltration and diafiltration mode. It is expected that diafiltration mode will become generally accepted for contributing the common techniques in this experiment.

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References

[1] K.G. Lee, A.E. Mitchel, T. Shibamoto, J. Agric. Food Chem. 48 (2000) 4817.
[2] A. Montano, A.H. Sanchez, A. De Castro, J. Food Sci. 65/6 (2000) 1022.
[3] A. Simon, L. Vandanjon, G. Levesque, P. Bourseau, Desalin. 144 (2002) 314.
[4] R.D. Noble, S.A. Stern, Membrane Separations Technology: Principles and Applications, Elsevier, Amsterdam, 1999, p. 394.
[5] M. Cheryan, Ultrafiltration and Microfiltration Handbook, Technomic Publishing Co., Lancaster, 1997.
[6] M. Cheryan, Ultrafiltration Handbook, Technomic Publishing Co., Lancaster, 1986, p. 206.
[7] A.S. Grandison, M.J. Lewis, Separation Processes in the Food and Biotechnology Industries: Principles and Applications, Technomic Publishing Co. Inc., Lancaster, 1996, p. 95.
[8] L. Wang, L. Song, J. Membr. Sci. 160 (1999) 41.
[9] H.E. Shallo, A. Rao, A.P. Ericson, R.L. Thomas, Preparation of Soy Protein Concentrate by Ultrafiltration, J. Food Sci. 66/2 (2001) 242.
[10] Salt Removal by Diafiltration [Brochure]. Ohio: Brochure of Amicon Corporation; 1981.
[11] Operating Manual DSS Lab Unit M20, Denmark: DSS; 2000.
[12] A.Y. Tamime, V.M.E. Marshall, Microbiology and Biochemistry of Cheese and Fermented Milk, Blackie Academic and Professional, London, 1997.