ULTRAFILTRATION OF LACTIC ACID BACTERIA (LAB) IN MUNG BEANS BROTH BY MIXED LAB CULTURE

(Ultrafiltrasi Bakteri Asam Laktat (BAL) Pada Kaldu Kacang Hijau Oleh Kultur BAL Campuran)

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ABSTRAK. *Pemekatan Bakteri Asam Laktat (BAL) dalam kaldu kacang hijau terfermentasi oleh kultur campuran Lactobacillus sp. dan Streptococcus thermophillus melalui ultrafiltrasi (UF) (20.000 MWCO) pada laju alir ~8,87 L/menit, suhu ruang serta tekanan 5 dan 7 bar selama 0, 30, 60, 90 dan 120 menit telah dilakukan guna mendapatkan jumlah BAL total optimal. Penelitian dilakukan untuk mendapatkan pengaruh tekanan dan waktu pemekatan terhadap kinerja membran UF (fluks, derajat pemekatan (DP) dan rejeksi solut) untuk menghasilkan produk probiotik dengan jumlah BAL total. Hasil penelitian menunjukkan bahwa tekanan dan waktu pemekatan berpengaruh terhadap kinerja membran UF serta total padatan, total protein dan jumlah BAL total. Lama waktu pemekatan menurunkan fluks dan meningkatkan DP, rejeksi pengamatan (R_{obs}) total padatan, R_{obs} total protein dan R_{obs} BAL total pada kedua tekanan. Waktu pemekatan optimal pada tekanan 5 bar dicapai selama 60 menit dengan menghasilkan permeat pada fluks 11,94 L/m^2.jam, konsentrasi total padatan 13,9423%, total protein 8,95%, jumlah BAL total 6,18 log CFU/mL, R_{obs} total padatan 3,45%, R_{obs} total protein 58,67%, R_{obs} BAL 100% dan DP 1,38 kali. Waktu pemekatan terbaik pada tekanan 7 bar dicapai selama 30 menit dengan menghasilkan permeat pada fluks 16,16 L/m^2.jam, total padatan 12,2879%, total protein 4,41%, jumlah LAB total 6,04 Log CFU/mL, R_{obs} total padatan 11,98%, R_{obs} total protein 45,76, R_{obs} BAL 99,5% dan DP 1,16 kali.*

Kata kunci: *Bakteri Asam Laktat (BAL), derajat pemekatan, kacang hijau (Phaseolus radiatus L.), membran ultrafiltrasi aliran melintang, probiotik*

ABSTRACT. *Increasing Lactic Acid Bacteria (LAB) concentration in fermented broth of mung beans by mixed culture of Lactobacillus sp. and Streptococcus thermophillus through ultrafiltration (UF) (20,000 MWCO) at flow rate of ~8.87 L/min, room temperature and pressure 5 and 7 bars for 0, 30, 60, 90 and 120 minutes was performed. The results showed that pressure and time affected on UF performance, total solids, total protein and total number of LAB. Optimal time at pressure 5 bar was reached 60 minutes with flux 11.94 L/m2.hour, total solids 13.9423%, total protein 8.95%, total LAB 6.18 log CFU/mL, R_{obs} of total solids 3.45%, total protein 58.67%, LAB 100% and DC 1.38 folds. The best time at 7 bar was reached 30 minutes with flux 16.16 L/m^2.hour, total solids 12.2879%, total protein 4.41%, total LAB 6.04 Log CFU/mL, Robs of total solids 11.98%, total protein 45.76%, LAB 99.5 and DC 1.16 folds.*

Keywords: *Cross-flow ultrafiltration (CFUF) membrane, Degree of Concentration (DC), Lactic Acid Bacteria (LAB), mung beans (Phaseolus radiatus L.), Probiotic*

1. INTRODUCTION

The functional characteristics of protein mung beans *(Phaseolus radiatus* L.*)* plays a larger role than nutritional considerations in determining their acceptability as ingredients in food systems (Agustine, 2010; Agustine, *et. al*., 2008; Agustine, *et. al*., 2006). One of the innovative food processes using mung bean as a raw material is preparation of probiotic mung bean *vegetable broth* extract. A typical preparation process of probiotic mung bean *vegetable broth* extract consists of pulverizing crude mung bean *vegetable broth* (Alice, 1989; Moerniati, 2009) by adding hot water and first homogenizing, first sieving, autoclaving, mixing and incubating by *Lactic Acid Bacteria (LAB*), second homogenizing and second sieving, concentrating via membrane and packaging (bottling). *LABs* are widely utilized in processes of food fermentation so that they might be exploited in *vegetable broth* of fermented mung bean. The end products of lactic fermentation confer the necessary protection against spoilage (acidification, bacteriocins), contribute to the desired flavors and add to the texture. The ability of different strains to produce these compounds is variable, and satisfactory levels are often achieved only by the use of mixed fermentations. During this fermentation process, sprouting of mung bean involves complex enzymatic reactions, which break down macromolecules (Goupry *et. al*., 2000; Qingli Z., *et. al*., 2011; LeBlanc, 2011). Fermentation broth products which are usually a complex mixture of components, heat sensitive and often low in concentrations have molecular weights (MW) in the range of 500-2,000 Dalton (Da.). It is typically able to separate solute molecules with MW from 1,000 to 1,000,000 Da. (1 to 100 nm) in size. *Lactobacillus bulgaricus* have a rod shape and size of cell particles in range of 1-10 μm (L) and 0.5-1.2 μm (W), while *Streptococcus thermophillus* have a ball shape with diameter < 1 μm. Certain microorganisms can use mono-, di- and oligosaccharides in medium and generate lactic acid during fermentation. Typical strains include *L. acidophilus, L. bulgaricus, L. delbrueckii, L. plantarum, Bifidobacterium longum, Leuconostoc mesenteroides, and S. thermophilus.* Some of these probiotics have produced pleasant organics to mask beany flavor in fermentation of soy milk or yield lactic acid in the soybean cooked syrup (Yuan, *et. al*., 1997; Aspiyanto, *at. al*., 2010).

In recent years, cross-flow ultrafiltration (CFUF) membrane-based technology is currently regarded as a new frontier, a most suitable techniques and a necessary process step of agro-food and dairy, biotechnology, biochemistry, pharmaceutical, medicine areas and chemical engineering and has been applied in concentrating, purifying or separating macromolecules (protein, polysaccharides), colloidally dispersed substances and suspended particles. Because of many unique properties of membrane technology, such as no phase change (low energy consumption), no chemical addition, and simple and mild operation and process, membrane process can easily be combined with other separation processes, minimize disposal problems, raise product recovery and purity, membrane process usually provide a better option over the traditional separation methods (Liyuang, *et. al*., 1999; Batt, *et. al.*, 1999).

UF membrane itself with linear dimension in the range of 1 to 100 nanometer (nm) and a thin surface skin of 0.1-1 micrometer (μm) supported by a porous substructure can retain solute molecules in the MW range 300-500.000 Da and most colloids based on the molecular size of the solute molecules and differences in the rates at which ions, molecules, or particles move cross the membrane. UF is undertaken at low operation pressure (< 10 bar) and osmotic pressure of the solute molecule is usually negligible. In general, separation using membranes may occur on the surface or within the porous matrix of the membrane (Tsapiuk, *et. al.*, 1993; Cheryan, 1986; Moerniati, 2009).

The goal of this experiment was to find out performance of UF membrane of 20,000 Molecular Weight Cut-Off (MWCO) or 20,000 Da. on concentration process of *Lactobacillus Acid Bacteria (LAB)* produced from mung beans *vegetable broth* fermented by mixed culture of *Lactobacillus* sp. and *S. thermophillus*. Performance of UF membrane included flux value, solute molecules rejection and degree of concentration (DC). Condition of UF operation was flow rate ~8.87 L/minute, room temperature (~25^{o}C) and pressure 5 and 7 bar for 0, 30, 60, 90 and 120 minutes, respectively.

2. METHODS

Materials used in this activity were crude mung beans *broth* (Research Centre for Chemistry-LIPI), inoculum of *LAB* isolated from mixed culture of *Lactobacillus* sp. and *S. thermophillus*, fresh water (RO water), skim milk, sugar, MRS (Man-Rogosa-Sharpe) agar (OXOID), chemical reagents (E. Merck), and commercial polysulphone (Psf) ultrafiltration (UF) membrane of 20,000 MWCO (GR-61-PP) purchased from Danish Separation System AS, Nakskov, Denmark. Main equipments utilized in this activity were pulverizer, series of *LAB* fermentation system in laboratory scale, homogenizer (Ultra-Turrax, Ika Labortechnik, T50, Jane & Kunkel, Germany), sieve of 140 mesh and 200 mesh (Retsch, Germany), autoclave (Cheng Yi, LS-50L, China), incubator, module membrane *Lab Unit M20* (DSS, Denmark) (DSS, 2000), Stopwatch (Hanhart Profil 2, Germany), instruments for chemical analyses and investigation of microbiology aspect of product.

Experimental Design

This experiment was performed by UF membrane of 20,000 MWCO at pump motor frequency of 25 Hz, room temperature (~25^{o}C) and operation pressure of 5 and 7 bar for 0, 30, 60, 90 and 120 minutes. Investigation of performance of UF membrane were flux value, solute molecules rejection on membrane and degree of concentration (DC) (Winston, *et. al*., 1992). Analyses were carried out on composition of extract of probiotic mung beans *vegetable broth* and retentate (concentrate) and permeate as a concentration process result consisting of total solids (Gravimetric method), total protein (Kjeldahl method), (A.O.A.C., 1990) and total *LAB* counts (Pour plate method) (Srikandi, 1989).

Preparation and Concentration of *LAB* Produced from Mung Beans *Vegetable Broth* Fermented by Mixed Culture of *Lactobacillus* sp. and *S. thermophillus* Using Ultrafiltration (UF) Membrane

Extract of mung bean *vegetable broth* which was produced subsequently through steps by adding hot water (± 80^{o}C, 7 parts) to crude mung bean broth (1 part), pulverizing and homogenizing (4,000 rpm, 15 minutes) and sieving via 200 mesh to yield supernatant I and non-soluble residue. Non-soluble residue was then added with hot water (± 80^{o}C, 1 part), homogenizing (2,000 rpm, 20 minutes) and sieving via 200 mesh to yield supernatant II and residue. Supernatant I and supernatant II was mixed to produce extract of mung bean *vegetable broth.* Extract of mung bean *vegetable broth* was autoclaved (121^{o}C, 15 minutes) and cooling to room temperature. Autoclaved extract of mung bean *vegetable broth* was subsequently added 15% of inoculum of *LAB* (*Lactobacillus* sp. and *S. thermophillus*), 10% skim milk and 12% of sugar and mixed by agitator and incubated (40^{o}C, 48 hours), homogenized (4000 rpm, 10 minutes) and sieved via 140 mesh to generate probiotic extract of mung beans *vegetable broth.* After the concentration process finish, UF membranes in module were thoroughly cleaned in place (CIP) by pumping a 1% sodium hydroxide solution at 3.5 L/minute, 60^{o}C and 3 bar for 30 minutes, followed by a rinse of pre filtered RO water. CFUF module used to concentrate of *LAB* in mung beans *vegetable broth* fermented by mixed culture of *Lactobacillus* sp. *and S.*

thermophillus was represented in Figure 1 (DSS, 2000).

Figure 1. Cross-Flow MF/UF/NF/HF or RO module used to concentrate of *LAB* in mung beans *vegetable broth* fermented by mixed culture of *Lactobacillus* sp. *and S. thermophillus* (DSS, 2000).

3. RESULT AND DISCUSSION

Characteristic of Probiotic Extract of Mung Beans *Vegetable Broth*

Crude mung bean *vegetable broth* produced from result of brine fermentation by inoculum of *Rhizopus*-C_1 with composition of 23% inoculum, 56% mung beans and 21% salt at room temperature for 20 weeks, probiotic mung beans *vegetable broth*, and inoculum of *LAB* isolated from mixed culture of *Lactobacillus* sp. and *S. thermophillus* was represented in Figure 2. Probiotic extract of mung beans *vegetable broth* is a product obtained via *LAB* fermentation using inoculum from mixed culture of *Lactobacillus* sp. and *S. thermophillus* on *vegetable broth* extract from mung beans. High contents of total protein (9.26%, dry weight basis), dissolved protein (7.8 mg/mL) and N-amino (4.2 mg/mL) will contribute its important role as source of savory flavor in which N-amino are amino acids and dissolved peptides becoming parameter of non-volatile compounds as source of savory flavor (Tamime, *et. al*., 1997). Total *LAB* counts (7.23 log CFU/mL) indicated that characteristic of this initial probiotic product contains biomass of vegetable protein in sufficient amount in which probiotic foods standard is > 6 log CFU/mL (Srikandi Fardiaz, 1989). The presence of reducing sugar (46.5 mg/mL) and salt (3.1595%) showed effect of brine fermentation process, while lactic acid as total acids (1.9503%) is metabolite products resulted by *LAB* during fermentation. Lactic acid is one of the metabolite products supporting functional properties and preservative components of probiotic product. Low content of total solids (13.4694%) demonstrated that this viscous liquid product like yoghurt has low content of fat (1.0769%) so that this product is save to be consumed as endemic beans-based functional foods (Tamime, *et. al*., 1997).

(a) (b) (c)

Figure 2. Crude mung bean *vegetable broth* produced from result of brine fermentation by inoculum of *Rhizopus*-C_1 with composition of 23% inoculum, 56% mung beans and 21% salt at room temperature for 20 weeks (a), probiotic mung beans *vegetable broth* (b) and inoculum of *LAB* isolated from mixed culture of *Lactobacillus* sp. and *S. thermophillus* (c).

Characteristic and composition of probiotic extract of mung beans *vegetable broth* which would be introduced as feed in concentrating by UF membrane consist of total solids of 13.4697%, total protein of 9.26% (dry weight), dissolved protein of 7.8 mg/mL, N-amino of 4.2 mg/mL, reducing sugar of 46.5 mg/mL, fat of 1.0769%, total acids of 1.9503%, salt of 3.1595%, and total *LAB* count of 7.23 log CFU/mL, respectively.

Effect of Concentration Process on Flux Value and Degree of Concentration (DC)

In concentration process using UF membrane (20,000 MWCO), the most important parameters are the flow rate of permeate (permeate flux), the observer rejection (R_{obs}) of membrane and degree of concentration (DC) or ratio of initial feed volume or weight to volume or weight remaining after UF. Flux (J) was determined by measuring the permeate volume (V) passing freely via a unit area of membrane (A) collected over the measured time interval (t), e.g. $J = V.A^{-1}.t^{-1}$ (Grandison, *at. al.*, 1996). The separation data of concentration process time and flux value obtained are presented in Figure 3, in which concentration process time is plotted against flux value for different operation pressures.At operation pressure of 5 bar, it takes place a sharply decline of the flux value (27.18 L/m^2.hour) within the first 0-30 minutes followed by a gradual decline at 30, 60, 90 and 120 minutes giving flux values at those times are of around 15.5, 11.94, 9.28 and 8.33 L/m^2.hour, respectively.

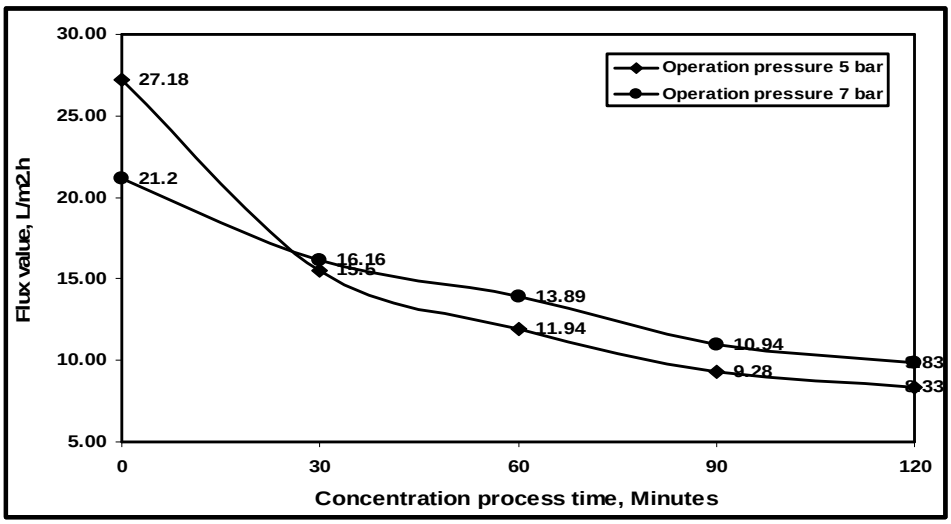


Figure 3. Effect of time on flux value as a result of concentration process of *LAB* produced from mung beans *vegetable broth* fermented by mixed culture of *Lactobacillus* sp. *and S. thermophillus* by UF membrane.

The similar trend or profile occurs also at operation pressure 7 bar in which a decrease of flux value occurs at the initial process (21.2 L/m^2.hour), followed by an decrease of flux value at 30, 60, 90 and 120 minutes which gave flux values at approximately of 16.16, 13.89, 10.94 and 9.83 L/m^2.hour. After 30 to 120 minutes of concentration process, the flux value at operation pressure of 7 bar was greater than that 5 bar. It can be said that an increase of operation pressure from 5 to 7 bar would affect on the flux value due to the presence of compaction in pores size of membrane. The drop of the flux value at flow rate of ~8.87 L/minute, room temperature (~25^{o}C), and pressure of 5 and 7 bar for all concentration process times were possibility caused by the solute molecules deposition at the top membrane surface and/or within the membrane (fouling) and interaction between driving force of fluid and sufficient high concentration of total solids (13.4694%) so

that it might increase fluid viscosity because of the presence of water mass removal as a consequent of flux value became more and more low. Only solute molecules having same or bigger MW than 20,000 MWCO would be retained at the top membrane surface and re-circulated into bulk fluid, while smaller size of solute molecules than 20,000 MWCO will pass freely through membrane as permeate. Increasing the flow rate increases turbulence and decreases the boundary layer thickness, and this rise the flux value. This reflects the higher porosity of UF membrane (20,000 MWCO), and higher porosity has a tendency forwards fouling. Fouling is a term generally used to describe the undesirable formation of deposits on membrane surface (Amjad, 1993). Based on the terminology according to IUPAC, fouling is a process yielding in loss of performance of a membrane because of the accumulation of dissolved or suspended substances on external surface, at its pore openings or within its pores (Koros, *et. al*., 1996).

Permeation rate (Flux) and separation factors (R_{obs} of solute molecule) are two key characteristic determining the performance of membrane. Both are influenced by composition factors, membrane physical and chemical properties and process conditions. Factors affecting rejection of UF membrane are shape and size of solute molecule, type of membrane material, membrane configuration, presence of other solutes, concentration of retained solute molecule, absorption of solute molecule by the membrane, and effect of microenvironment (Grandison, *at. al*.; O'Sullivan *et. al*., 1984).

UF data presented in terms of degree of concentration (DC) or volume concentration ratio (VCR) is defined as ratio of original feed volume divided by volume remaining after UF or ratio of original feed volume to original feed volume minus collected permeate volume (assuming no losses) is expressed as DC or $VCR = V_f/V_c = V_f/(V_f - V_p)$, where V_f is initial feed volume, V_c is retentate (concentrate) and V_p is permeate volume at interval time. As soon as DC exceeds 1 (one), volume of permeate will exceed that of the retentate (concentrate). DC may range from as low as 1.5 folds for some viscous fluids, to up to 50 folds for dilute protein solutions. Higher DC are used for UF than for reverse osmosis (RO), e.g. up to 25-30 folds for UF of cheese-whey, compared to 5 folds for RO of cheese-whey (Scott, 1998). Effect of time on DC as a result of concentration process of *LAB* produced from mung beans *vegetable broth* fermented by mixed culture of *Lactobacillus* sp. and *S. thermophillus* under flow rate of ~8.87 L/minute, room temperature (~25^{o}C) and pressure of 5 and 7 bar was shown in Figure 4. At the same time, it would occurred an increase of DC (1, 1.16, 1.38, 1.46 and 1.65 folds), as presented in Figure 5. This increase of DC was caused by much amount of water mass passing via the membrane so that solute particles will be retained at the membrane surface during concentration process. Selection ability on UF membrane in separating solutes molecules with suitable MWCO, high performance of products and pH tolerance on wide operation conditions enables occurs a better separation process. The ability of the UF membrane to selectively concentrate macromolecules leads to processing problems. The greatest effect with respect to processing biological product is an increase in viscosity with concentration process (O'Sullivan, *et. al*., 1984). The whole conditions, UF membrane process under pressure 5 bar gave higher DC than that 7 bar during concentration process. At the similar time, DC became more and more low (1, 1.16, 1.3, 1.46 and 1.65 folds), as demonstrated in Figure 4.

Effect of Concentration Process Condition on Observer Rejection Coefficient (R_{obs}) of Total Solids, Total Protein and Total *LAB* Count

The term rejection coefficient (R) is used to describe the action of the membrane or any deposit on the membrane in preventing passage of a solute molecule of given molecular size. Observer rejection

coefficient (R_{obs}) is one of the convenient measures of the selectivity for all pressure driven processes-based membrane. R_{obs} of any solute molecule is defined as $(C_f - C_p)/C_f = 1 - (C_p/C_f)$, where C_f is concentration of solute molecule in feed and C_p is concentration of solute molecule in permeate. R_{obs} values normally range between 0 and 1, but it is sometimes expressed as percentages (0-100). The ideal membrane will retain all solute molecules than its cut-off (C_p=0) and allow the smaller ones to pass freely (C_p=C_f). It is likely that R_{obs} is not constant but changes with concentration of retentate (concentrate). Observer rejection coefficient (R_{obs}) is determined experimentally for each solute molecule in the feed, by sampling feed and permeate at the same time and analyzing that solute molecule. The separation data of concentration process time and R_{obs} of total solids, R_{obs} of total protein and R_{obs} of total *LAB* count are shown in Figure 6.

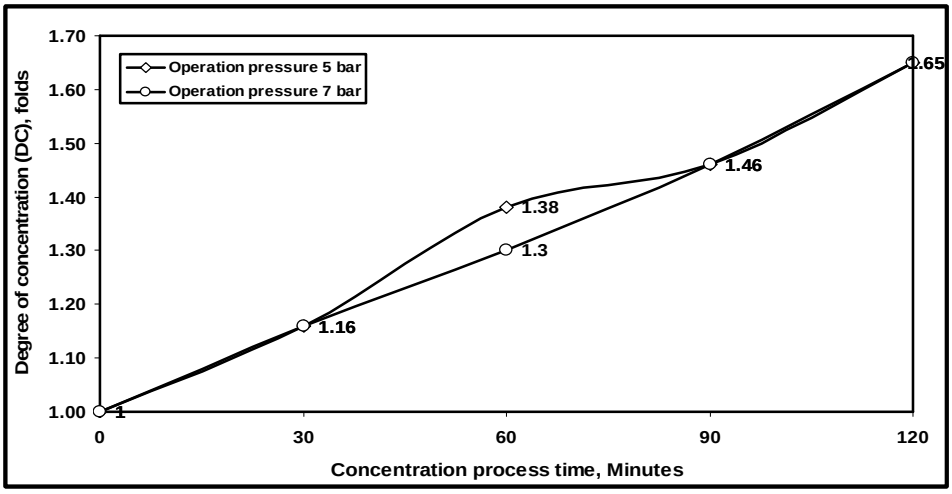


Figure 4. Effect of time on degree of concentration (DC) as a result of concentration process of *LAB* produced from mung beans *vegetable broth* fermented by mixed culture of *Lactobacillus* sp. *and S. thermophillus* by UF membrane.

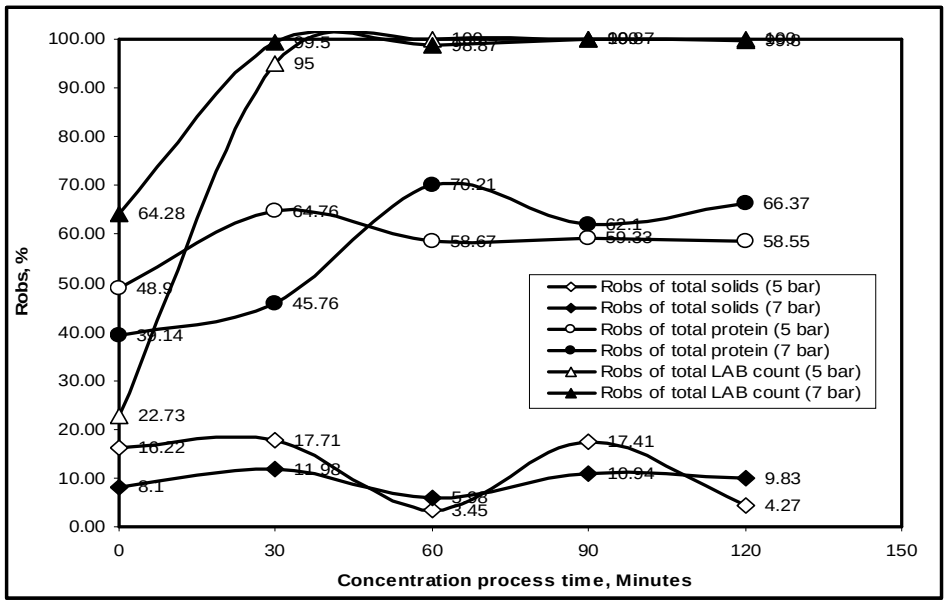


Figure 5. Relationship between time, and R_{obs} of total solid, R_{obs} of total protein and R_{obs} of total *LAB* count by UF membrane.

The separation data of concentration process time and Composition of components in permeate and retentate to concentrate *Lactobacillus Acid Bacteria (LAB)* produced from mung beans *vegetable broth* fermented by mixed culture of *Lactobacillus* sp. and *S. thermophillus* via UF membrane at flow rate of ~8.87 L/minute), room temperature (~25^{o}C) and operation pressure 5 bar and 7 bar for 0-120 minutes are shown in Table 1.

Table 1. Composition of components in permeate and retentate (concentrate) from *Lactobacillus Acid Bacteria (LAB)* produced from mung beans *vegetable broth* fermented by mixed culture of *Lactobacillus* sp. *and S. thermophillus* via UF membrane at flow rate of ~8.87 L/minute), room temperature (~25^{o}C) and pressure 5 and 7 bar for 0-120 minutes.

Kind of material, pressures and Components		Concentration process time (minutes)				
		0	30	60	90	120
Total Solids (%)	Permeate-5 Bar	12.381	12.5012	13.4615	14.2064	12.6214
	Retentate-5 Bar	14.7787	15.1916	13.9423	14.8407	15.2816
	Permeate-7 Bar	12.2541	12.2879	13.7122	12.8944	14.5282
	Retentate-7 Bar	14.1038	13.3396	14.5852	14.6498	14.6617
Total Protein (%, dry weight)	Permeate-5 Bar	3.94	3.04	4.43	2.67	3.01
	Retentate-5 Bar	7.71	7.50	8.95	9.14	8.87
	Permeate-7 Bar	3.98	4.41	2.77	3.79	3.36
	Retentate-7 Bar	6.54	8.13	9.30	10.00	9.99
Total *LAB* count (log CFU/mL)	Permeate-5 Bar	6.82	5.04	0	0	0
	Retentate-5 Bar	6.34	6.34	6.18	6.46	6.04
	Permeate-7 Bar	6.48	6.04	6.41	6.29	6.91
	Retentate-7 Bar	6.92	8.30	8.36	8.18	8.62

Rejection of Total Solids (R_{obs} of Total Solids)

At operation pressure of 5 bar occurred subsequently a gradually increase of R_{obs} of total solids in the time range 0 to 30 minutes (10.22-17.71%), a decrease in the time range 30 to 60 minutes (17.71-3.45%), an increase in the time range 60 to 90 minutes (3.45-17.41%) and a drop to the end concentration process (4.27%). Whereas, at operation pressure of 7 bar showed a more stable of R_{obs} of total solids to final concentration process (4.27%). For both operation pressures for time range of 0-120 minutes displayed low R_{obs} of total solids. This result of R_{obs} of total solids was caused by much more total solids passing across UF membrane (20,000 MWCO) than that total solids retained in membrane surface and bulk fluid (retentate or concentrate) in feed tank. The overall concentration processes and times, at operation pressure of 5 bar gave higher rejection of total solids than that operation pressure of 7 bar, except for 60 and 120 minutes of concentration processes. This matter was estimated that at operation pressure of 7 bar for 60 and 120 minutes was generated a sufficient high driving force, so it might be able to pass freely total solids particles to the permeate side. Nevertheless, UF membrane system was generally able to concentrate 30% of the whole materials. Many fermentation products possessing solute molecule with molecular sizes smaller than the membrane's cut-off limit (20,000 MWCO) or pore size can pass via the pores of membrane.

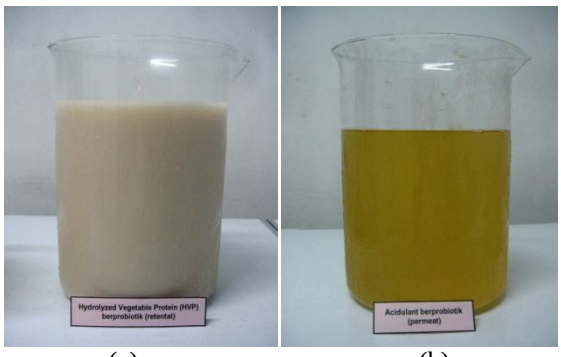
Separation factor (R_{obs} of solute molecule) is one of the key characteristics in determining the performance of membrane. It is influenced by shape and size of solute molecule, type of membrane material, membrane configuration, presence of other solutes, concentration of retained solute molecule, absorption of solute molecule by the membrane, and effect of microenvironment (Grandison, *et. al.* 1996; O'Sullivan, *et. al*., 1984).

Rejection of Total Protein (R_{obs} of Total Protein)

As shown in Figure 5, difference in operation pressure and long time of concentration process would also rise proportionally R_{obs} of total protein at operation pressure of 5 bar in the time range of 0 to 30 minutes (48.9-64.76%) followed by a gradually drop in the time range of 30 to 120 minutes (64.76-58.55%). While, under operation pressure of 7 bar in the time range of 0 to 60 minutes (39.14-70.21%) occurs subsequent a proportionally increase of R_{obs} of total protein, a decline in the time range of 60 to 90 minutes (70.21-62.1%) and a rise in the time range of 90 to 120 minutes (62.1-66.37%). From this time range, R_{obs} of total protein reached had showed that a difference concentration of total protein in retentate (concentrate) and permeate was not adequate high, but concentration of total protein in retentate (concentrate) was higher than that in permeate. It can be said that UF membrane (20,000 MWCO) was sufficient able to separate and reject this component in retentate (concentrate). Long time of concentration process, performance of UF membrane (20,000 MWCO) indicated a drop of ability in retaining total protein, in which at operation pressure of 7 bar after 60 minutes and at operation pressure of 5 bar after 30 minutes, R_{obs} of total protein became more and more low. With higher operation pressure caused higher driving force, so much more total protein would pass freely to permeate side. Total proteins are the accumulation of all soluble and non-soluble peptides in feed (bulk solution), and were one of the important parameters of probiotic products, as well as were a reference on intensity of savory taste in *vegetable broth* products. In other words, they were the source of amino acids as savory (umami) precursor (Agustine, *et. al.*, 2006). R_{obs} of total protein is affected by rather broad membrane pore size distribution, protein-protein interactions (e.g. protein aggregation), protein-membrane interactions (e.g. protein adsorption within the porous structure of membrane and fouling), and concentration polarization) (Crespo, *et. al.*, 1999).

Rejection of Total *LAB* Count (R_{obs} of Total *LAB* Count)

As represented in Figure 6, long time of concentration process would result a high R_{obs} of total *LAB* count. This high R_{obs} of total *LAB* count was caused by a successfully membrane separation in concentrating *LAB*. In other words, total *LAB* count in retentate was higher than that in permeate or separation efficiency of *LAB* nearly 100%.



(a) (b)

Figure 6. Retentate (concentrate) (a) and permeate (b) as a result of concentration of *LAB* produced from mung beans *vegetable broth* fermented by mixed culture of *Lactobacillus* sp. *and S. thermophillus* via UF membrane at flow rate of ~8.87 L/minute, room temperature (~25^{o}C) and operation pressure of 5 bar for 60 minutes.

Long time of concentration process at operation pressure of 5 bar for 0, 30, 60, 90 and 120 minutes gave R_{obs} of total *LAB* count of 22.73, 95, 100, 100 and 100%, respectively, while R_{obs} of total *LAB* count achieved at operation pressure of 7 bar for

0, 30, 60, 90 and 120 minutes were 64.28, 99.5, 98.87, 99.87 and 99.8%, respectively. For all concentration processes, at operation pressure of 5 bar gave higher R_{obs} of total *LAB* count than that operation pressure of 7 bar because increase of operation pressure from 5 to 7 bar on *LAB* cells are not able to cross an UF membrane barrier. At R_{obs} of total *LAB* count of 100% indicated that UF membrane (20,000 MWCO) had operate ideally in concentrating *LAB* cells on the membrane surface. On the other hand, *LAB* cells with smaller molecular sizes than the membrane's cut-off limit or membrane pore size will pass freely through the membrane. This matter occurs due to their large pores size of UF membrane in the range of 1 to 100 nm. Type of *LAB* cells, such as *S. thermophillus*, are ball shape with diameter of ≤ 1 μm, whereas another type of *LAB* cells, such as *L. bulgaricus*, are cylindrical form in the range of 1-10 μm (W) x 0.5-1.2 μm (L) (Batt, *et. al*., 1999), so *LAB* cells having larger size than pores size of UF membrane (20,000 MWCO) enables occurrence an accumulation of *LAB* cells on the top membrane surface as retentate (concentrate) and only less *LAB* cells pass freely through membrane as permeate.

4. CONCLUSIONS

Flux and rejection are key factors for evaluating membrane performance. Flux indicates the amount of permeate passed via membrane, while rejection represents how much solids have been removed by membrane. These two factors show capability of the membrane's solid removal. Relation between flux and degree of concentration (DC) showed a contrary condition in which more and more high flux will accelerate solute particles to pass freely through the membrane so that DC will be low. Long time of concentration at flow rate ~8.77 L/minute, room temperature (~25^{o}C) and pressures 5 and 7 bar was tend to affect flux, degree of concentration (DC), rejections of total solids (R_{obs} of total solids), R_{obs} of total proteins, R_{obs} of total *LAB*. Interactions between pressures 5 and 7 bar and long time of concentration would decrease flux, and increase DC and retentate (concentrate) composition (total solids, total protein and total *LAB* counts). Based on performance of UF membrane (20,000 MWCO), optimal time of concentration at flow rate of ~8.87 L/minute, room temperature (~25^{o}C) and pressure 5 bar to produce retentate (concentrate) as probiotic savory flavor was reached for 60 minutes with R_{obs} of total *LAB* 100%. While, the best time of concentration to yield permeate for probiotic acidulant as side product was reached for 30 minutes. At the best performance of this UF membrane (20,000 MWCO) was produced permeate with flux of 11.94 L/m^2.hour and retentate (concentrate) as probiotic savory flavor with concentrations of total solid of 13.9423%, total protein of 8.95% and total *LAB* count of 6.18 log CFU/mL, and R_{obs} of total solids of 3.45%, R_{obs} of total protein of 58.67% and R_{obs} of total *LAB* counts of 100%, while degree of concentration (DC) of 1.38 folds. Whereas, the best time of concentration to produce permeate as probiotic acidulant (by product) with total solid concentration of 12.2879% and total *LAB* count of 1.01 x 10^7 cfu/mL was reached for 30 minutes.

REFERENCES

Alice, H. L. (1989). Lactic Acid Bacteria and Intestinal Drug and Cholesterol Metabolism, in Seppo Salminen, Atte von Wright and Arthur Ouwehand. *Lactic Acid Bacteria: Microbiological and Functional Aspects* (3 ed.). New York: Marcell Dekker Inc.

Agustine, S. (2010). Pengaruh Aktivitas Proteolitik *Aspergillus* sp-K3 Dalam Perolehan Asam-asam Amino Sebagai Fraksi Gurih Melalui Fermentasi Garam Pada Kacang Hijau *(Phaseolus radiatus* L.*). Majalah Pangan*, 19(1), 81-82.

Agustine, S., Aspiyanto, Hakiki, M. & Yati, M. (2008). The Effect of Process Conditions in Preparation of *Vegetable Broth* as Savory Flavor From Mung Beans Using Inoculum of *Rhizopus*-C1. *Indonesian Journal of Chemistry*, 8(8), 363.

Agustine, S. & Aspiyanto. (2006). Pembuatan Konsentrat Kacang Hijau (*Phaseolus radiatus* L.) Terfermentasi Sebagai Probiotik Ingredient Melalui Membran Osmosa Balik. *Prosiding Seminar Nasional Biologi*. Cibinong: Pusat Penelitian Bioteknologi – LIPI.

Aspiyanto & Agustine, S. (2010). Effect of Diafiltration on Preparation of Fermented Mung Beans Concentrate as Probiotic Savory Flavor Through Ultrafiltration Membrane, *Jurnal Makara Seri Teknologi*, 14(2), 77.

A.O.A.C. (1990). *Official Methods of Analysis of the Association of Analytical Chemistry*. Washington D.C.: A.O.A.C. Inc.

Batt, C. A., Robinson, R. K., & Patel, P. D. (1999). *Encyclopedia of Food Microbiology*. New York: Academic Press.

Cheryan, M. (1986). *Ultrafiltration Handbook*. U.S.A.: Technomic Publishing Company Inc.

Danish Separation Systems. (2000). *Operating Manual DSS LabUnit M20*. Denmark: Danish Separation Systems AS.

E. A. Tsapiuk & M. T. Bryk. (1993). An Interpretation of The Separation of Low and High Molecular Weight Solutes by Ultrafiltration. *Journal of Membrane Science*, 79, 227–240.

Grandison, A. S. & Lewis, M. J. (1996). *Separation Processes in the Food and Biotechnology Industries: Principles and Applications* (1 ed.). Lancaster: Technomic Publishing Co. Inc.

Connell, H., Zhu, J., & Bassi, A. (1999). Effect of Particle Shape on Cross-flow Filtration Flux, *Journal of Membrane Science*, 153, 121.

Yeh, H. M. & Cheng, T. W. (1999). Analysis of The Slip Effect on The Permeate Flux in Ultrafiltration Membrane. *Journal of Membrane Science*, 154, 41.

Le Blanc, J. G., Laiño, J. E., del Valle, M. J., Vannini, V., van Sinderen, D., Taranto M. P., de Valdez, G.F., de Giori, G. S., & Sesma, F. (2011). B-Group Vitamin Production by Lactic Acid Bacteria – Current Knowledge and Potential Applications. *Journal of Applied Microbiology*, 111(6), 1297.

Crespo, J. P. S. G., Trotin, M., Hough, D., & Howell, J. A. (1999). Use of Fluorescence Labeling to Monitor Protein Fractionation by Ultrafiltration Under Controlled Permeate Flux. *Journal of Membrane Science*, 155, 210.

Scott, K. (1998). *Handbook of Industrial Membranes* (2 ed.). Oxford: Elsevier Advanced Technology.

Liyuang, W., & Lianfa, S. (1999). Flux Decline in Crossflow Microfiltration and Ultrafiltration: Experimental Verification of Fouling Dynamics. *Journal of Membrane Science*, 160, 41.

Moerniati. (2009). Seasoning Berprobiotik: Inovasi Fungsional Savory dari Kacang Merah *(Phaseolus vulgaris* L.*)* Terfermentasi oleh *Rhizopus*-PL19 Melalui Mikrofiltrasi, *Majalah Pangan*,54(18), 68-80.

Moerniati, S., Agustine, S. & Aspiyanto. (2009). Potensi Nanofiltrasi dalam Pemekatan Bakteri Asam Laktat Sebagai Probiotik Savory dari Kacang Hijau *(Phaseolus radiatus* L.*)* Terfermentasi oleh *Rhizopus*-C_1. *Jurnal Teknologi Pertanian AGRITECH*, 29(3), 146.

O'Sullivan, T. J., Epstein, A. C., Korchin, S. R. & Beaton, N. C. (1984). Applications of Ultrafiltration in Biotechnology. *Chem Eng. Process*, 80, 68-75.

Qingli, Z., Ren, J., Zhao, H., Zhao, M., Xu, J. & Zhao, Q. (2011). Influence of Casein Hydrolysates on The Growth and Lactic Acid Production of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus. International Journal of Food Science & Technology*, 46(5), 1014.

S. Goupry, Rochut, N., Robins, R.J. & Gentil, E. (2000). Evaluation of Solid-Phase Microextraction for the Isotopic Analysis of Volatile Compounds Produced during Fermentation by Lactic Acid Bacteria. *Journal of Agricultural and Food Chemistry*, 48(6),. 2222-2227.

Srikandi, F. (1989). *Penuntun Praktek Mikrobiologi Pangan.* Bogor: IPB Press.

Tamime, A. Y. & Marshall, V. M. E. (1997). Microbiology and Technology of Fermented Milks. in B. A. Law (Ed.),

Microbiology and Biochemistry of Cheese and Fermented Milks (2 ed.). London: Blackie Academic and Professional.

W. J. Koros, Ma, Y. H., & Shimidzu, T. (1996). Terminology for Membranes and Membrane Process. *Journal of Membrane Science*, 120, 149.

Winston Ho, W. S., & Sirkar, K. K. (Eds.). (1992). *Membrane Handbook*. New York: Van Nostrand Reinhold.

Yuan-Kuang, G., Chiu-Hsia, C., & Jing-Kun, Y. (1997). Processing of Soybean Soaking Water with a NF – RO Membrane System and Lactic Acid Fermentation of Retained Solutes. *Journal of Agricultural and Food Chemistry*, 45, 4096.