SEPARATION OF SAVORY FRACTION FROM AUTOLYSATE OF KIDNEY BEAN (*Phaseolus vulgaris* L.) FERMENTED BY *Rhizopus* sp.-PL19 THROUGH CROSS-FLOW MICROFILTRATION (CFMF) MEMBRANE MODULE

PEMISAHAN FRAKSI GURIH DARI AUTOLISAT KACANG MERAH (*Phaseolus vulgaris* L.) TERFERMENTASI OLEH *Rhizopus* sp.-PL19 MELALUI MODUL MEMBRAN MIKROFILTRASI CROSS-FLOW (MFCF)

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ABSTRACT

Separation of savory fraction from autolysate of kidney bean (*Phaseolus vulgaris* L.) fermented by *Rhizopus* sp.-PL19 as potential seasoning agent was performed using cross-flow microfiltration (CFMF) technique in module scale. The goal of this experiment was to find out the effect of pressure and time on performance of MF membrane, and compositions of retentate and permeate. The result of experiment showed that separation and/or purification using CFMF technique gave permeate as savory fraction with better clearness and sharper aroma than retentate, but retentate had better composition than permeate. During separation increased total protein, dissolved protein and salt concentrations, but decreased total solid, reducing sugar and fat concentrations in permeate at pressure of 2 and 4 bar. Based on N-Amino concentration in permeate, the optimum condition of separation was reached at pump motor frequency of 10 Hz and room temperature with pressure of 2 and 4 bar for 10 minutes. This condition produced permeate with fluxes of 51.11 and 52.55 L/m².hour, and concentrations of N-Amino of 5.50 and 9.80 mg/mL, dissolved protein of 1.01 and 0.97 mg/mL, total protein of 4.85 and 12.10 % (dry weight), reducing sugar of 55.75 and 53.75 mg/mL, salt of 1.16 and 1.06 %, fat of 0.41 and 0.65 %, and total solids of 7.82 and 6.35 %, respectively.

Keywords: Autolysate, kidney bean (*Phaseolus vulgaris* L.), *Rhizopus* sp.-PL19, cross-flow microfiltration (CFMF), permeate, retentate.

ABSTRAK

Pemisahan fraksi gurih dari autolisat kacang merah (*Phaseolus vulgaris* L.) terfermentasi oleh *Rhizopus* sp.-PL19 sebagai bahan seasoning potensial dilakukan menggunakan teknik mikrofiltrasi cross-flow (MFCF) pada modul. Penelitian bertujuan untuk mencari pengaruh tekanan dan waktu pemisahan terhadap kinerja membran mikrofiltrasi

(MF) dan komposisi dalam retentat dan permeat. Hasil penelitian menunjukkan bahwa pemisahan dan/atau pemurnian menggunakan teknik MFCF memberikan permeat sebagai fraksi gurih savory dengan kejernihan lebih baik dan aroma lebih tajam daripada retentat, tetapi retentat memiliki komposisi lebih baik daripada permeat. Pemisahan dengan waktu lama menaikkan konsentrasi total protein, protein terlarut dan garam, namun mengurangi konsentrasi total padatan, gula pereduksi dan lemak dalam permeat pada tekanan 2 dan 4 bar. Berdasarkan konsentrasi N-Amino dalam permeat, kondisi optimum pada pemisahan dicapai dibawah frekuensi motor pompa 10 Hz dan suhu ruang dengan tekanan 2 dan 4 bar selama 10 menit. Kondisi ini menghasilkan permeat dengan fluks masing-masing 51,11 dan 52,55 L/m².jam serta konsentrasi N-Amino of 5,50 dan 9,80 mg/mL, protein terlarut 1,01 dan 0,97 mg/mL, total protein 4,85 dan 12,10 % (berat kering), gula pereduksi 55,75 dan 53,75 mg/mL, garam 1,16 dan 1,06 %, lemak 0,41 dan 0,65 % serta total padatan 7,82 dan 6,35 %.

Kata kunci : Autolisat, kacang merah (*Phaseolus vulgaris* L.), *Rhizopus* sp.-PL19, mikrofiltrasi cross-flow (CFMF), permeat, retentat.

INTRODUCTION

One of the most important processes occurring during the aging of kidney bean (*Phaseolus vulgaris* L.) broth in contact with lysis of cells is autolysis, which releases substances that exert a major impact on the sensory characteristics of the kidney bean broth. Autolysate of kidney bean broth via brine fermentation by *Rhizopus* sp.-PL19 is a base ingredient suspension produced by solving cell components with enzymatic activity via thermal process at a certain pH value to increase glutamic acid as savory (umami) fraction⁽¹⁾. Autolysis on miso analogue kidney bean broth or taucu

is performed in order to kill activated vegetative cells to increase amino acids as umami source and substrate alternative with specific savory flavor ⁽²⁾. Use of *Rhizopus* sp.-PL19 from isolation of *laru tempeh* is a development of inoculum application used in preparation of tempeh until now ⁽³⁾. Kidney bean is selected to diversify processed food, utilize less useful local beans and increase economic added value. In development, this autolysate can be applied in snack, soup and dressing salad products, such as natural flavor product ⁽⁴⁾.

Separation of savory fraction from autolysate of kidney bean *broth* through CFMF technique is needed to separate amino acids as savory fraction from other solid components utilized as a non-volatile component for development of a savory flavor product. CFMF technique is selected due to the use of low temperature during operation, no the use of chemical treatments, such as solvents, and a significant amount of proteinaceous solids, flavors and flavor precursor desirable in a autolysate concentrate ⁽⁵⁾. The CFMF technique is a solvent-free, non-thermally abusive, fat removal and solids separation technique. Microfiltration (MF) is effectively able to separate and larger macromolecules than 500,000 Molecular Weight Cut-Off (MWCO) or particle in range 0.1 – 10 µm, fat (1 – 10 µm), protein (0.04 – 2 µm) and sugar (8 – 20 µm), and passes taste, aroma and flavor compounds (< 0.04 µm), color pigment (0.1 – 10 µm), monosaccharides (0.001 µm), peptides (0.01 – 0.1 µm) and salt (0.001 – 0.01 µm) via membrane at low pressure of up to about 6 – 8 bar ^(6,7-8).

The goal of this experiment was to find out the effect of pressure and time on separation product of autolysate of kidney bean *broth* as a result of brine fermentation by *Rhizopus* sp.-PL19 via 0.2 µm MF membrane at pump motor frequency of 10 Hz and room temperature.

MATERIALS AND METHODS

Materials and Equipments

Main materials used in this work were autolysate of kidney bean *broth*, *Rhizopus* sp.-PL19 (Research Centre for Chemistry - Indonesian Institute of Sciences), analytical grade chemical reagents purchased from local market and 2 sheets of commercial Microfiltration (MF) membrane of 0.2

µm (GRM-0.2-PP) with diameter 20 cm and effective membrane area of 0.036 m² (Danish Separation Systems AS, Nakskov, Denmark). Main equipments utilized in this work were local pulverizing machine, shaker (Memmert, Germany), homogenizer (Ultra Torrax, Germany), sieve of 200 mesh (Retsch, Germany), autoclave (Cheng Yi, LS-50L, China), incubator, series of LAB fermentation system in laboratory scale, MF/UF/NF/RO Membrane Module (LabUnit M20, Danish Separation Systems AS, Nakskov, Denmark) equipped by Positive Displacement Pump Rannie 25.38 with cross-flow rate of 3.5 – 15 L/minute and maximum operation pressure of 50 bar, salinometer (ATAGO), and Spectrophotometer UV-1201 instruments.

Experimental Design

These experiments were conducted through processes of autolysis and separation using 0.2 µm MF membrane. Investigation was carried out on permeate volume passing via area unit of MF membrane at pump motor frequency of 10 Hz, room temperature and pressure of 2 and 4 bar for 0, 5, 15, 20 and 25 minutes, respectively. Analysis conducted on the products of autolysate, autolysate feed passing 200 mesh, and separation process (permeate and retentate) were total solids (Gravimetric method), total protein (Kjeldahl), dissolved protein (Lowry), reducing sugar (Somogyi-Nelson), salt (Salinometer), fat (Soxhlet) ⁽⁹⁾ and N-Amino (Cu) ⁽⁹⁾.

Process steps

Autolysis process of kidney bean *broth* and separation of autolysate of kidney bean *broth* through CFMF technique.

Preparation and separation of autolysate suspension via 0.2 µm MF membrane were schematically represented in Figure 1. Two parts of fermented kidney bean *broth* was pulverized by adding 3 parts of fresh water in pulverizing machine, adjusted pH value by adding NaOH/HCl to 5.5, heated in shaker at ± 50 °C, homogenized at 140 rpm for 36 hours, and inactivated at 70 °C for 5 minutes, so it is produced autolysate of kidney bean. This autolysate of kidney bean (1 part) was dissolved by adding 4 parts of fresh water to total solids approximately 5 %, homogenized using homogenizer at 3,000 rpm for 15 minutes, and filtered via High Separator Frequency with sieve of 200

mesh, so it is resulted autolysate suspension filtrate and residue. This autolysate suspension filtrate was used as a feed in separation process via 0.2 μ m MF membrane.

The feed tank of the membrane module was filled with the autolysate suspension filtrate to about one-third capacity (3,500 mL). The feed of autolysate suspension filtrate in feed tank is subsequent pumped tangentially by means of a fixed pump motor frequency of 10 Hz (cross-flow rate of $\sim$ 3.5 L/minute) through a pre-filtered in 200 μ m filter, heat exchanger system, and into a membrane module (plate & frame) mounted vertically at pressure of 2 bar for 5 minutes until the permeate yielded was yellow clear liquid. While, the rejected component in feed, which is not passed through the membrane, is re-circulated continuously to the feed/retentate/bulk solution tank as retentate to keep an effectively constant concentration. Temperature in feed/retentate tank is controlled and kept constant at $\sim$ 23 - 25 $^{\circ}$ C (room temperature) using chiller flowed by tap water.

Figure 1. Flow diagram of autolysis process of kidney bean broth and separation of savory fraction from autolysate through 0.2 μ m MF membrane.

During a 180 minutes period, sampling is carried out on the retentate in feed/retentate tank and permeate every 5 minutes (0, 5, 15, 20 and 25 minutes, respectively). After the separation finish, membranes in module were thoroughly cleaned in place by pumping a

1 % sodium hydroxide solution at 3.5 L/minute, 60 $^{\circ}$ C and 2 bar for 30 minutes, followed by a rinse of pre filtered nano pure water. With the similar condition, separation of autolysate suspension was carried out via CFMF technique at pressure of 4 bar⁽¹⁰⁾.

RESULTS AND DISCUSSION

Characteristic of autolysate of kidney bean and autolysate feed

Result of autolysates of kidney bean broth by *Rhizopus* sp.-PL19 and autolysate sieved via 200 mesh is represented in Table 1. From autolysate composition, valuable components in savory fraction were total protein (11.43 %, dry weight), dissolved protein (6.5 mg/mL) and amino acids as N-amino (12.6 mg/mL). Other components, such as reducing sugar (375 mg/mL), fat (0.43 %) and salt (2.97 %), were supporting components in the whole tastes, while total solids (24.20 %) indicated total components produced during autolysis process. This savory fraction composition of autolysate is not only influenced by initial protein concentration in crude broth, but also by proteolytic activity of *Rhizopus* sp.-PL19 and condition factors of autolysis. Autolysis at 50 $^{\circ}$ C and pH 5.5 for 36 hours causes its occurrence of substrate protein hydrolysis, namely a mixture between kidney bean, salt and inoculum of *Rhizopus* sp.-PL19 and rice substrate fermented by using fungal protease enzyme for 18 weeks. At this condition, possibility of protein matter contribution from cell body by lysis of fungal is small, because during brine fermentation has also undergone a lysis, such as brine fermentation in preparation of moromi⁽¹¹⁾ and spore with vegetative form which is still present. Although, microorganisms growing during fermentation give also protein contribution. Concentrations of amino acids as N-amino and dissolved protein are proteolytic activity product of *Rhizopus* sp.-PL19 to hydrolyze protein substrate, in which 36 hours of fermentation enables to optimize this activity⁽¹²⁾. Dissolved protein is all of dissolved peptides and amino acids in water and indicates its occurrence of hydrolysis by protease enzyme of fungal to form amino acids, especially glutamic acid as non-volatile savory flavor precursor.

Autolysate pulverizing at an autolysate-to-fresh water ratio of 1 part : 4 parts followed by homogenizing 3,000 rpm for 15 minutes and sieved through 200 mesh

in order to get a product used in CFMF technique with smaller size of particles to decrease a fouling on membrane surface. This process causes its occurrence of drop on concentration of total solid which is lower than initial concentration of autolysate (24.20 %). Total solids are parameter for all components in autolysate, which will affect on CFMF technique, especially permeate flux value and flow rate of autolysate suspension. Similar case occurs on fat concentration showing a higher increase than autolysate (0.64 %). This matter is possibility caused by its occurrence of suspension emulsification by homogenizing process at 3,000 rpm for 15 minutes, so that fat matter can be extracted maximally.

Table 1. Result of autolysates of kidney bean *broth* by *Rhizopus* sp.-PL19 and autolysate passing 200 mesh [used as feed in CFMF technique].

Type of material	Composition						
	Total Solid %	Total Protein %	Dissolved Protein mg/mL	N-Amino mg/mL	Reducing Sugar %	Fat %	Salt %
Autolysate of kidney bean <i>broth</i> by <i>Rhizopus</i> sp.-PL19	24.20	11.43	6.50	12.60	375	0.43	2.97
Autolysate passing 200 mesh [Feed in CFMF]	8.81	9.23	1.11	5.60	74.75	0.63	1.43

Both these type of autolysates appearance a physical different, in which autolysate is brown suspension, thick and savory (umami) taste, whereas autolysate used as feed in CFMF technique is more aqueous suspension, as presented in Figure 2. Autolysate color relates with type of kidney bean, brine fermentation and autolysis process causing its occurrence of Maillard reaction to form brown pigment of melanoidin, as a consequence of reaction result between amino acids and sugar through long time of autolysis and heating (50 °C) ⁽¹³⁾.

Figure 2. Autolysate of kidney bean *broth* via brine fermentation by *Rhizopus* sp.-PL19 at room temperature, autolysis at 50 °C and pH 5.5 for 36 hours, and sieve of 200 mesh used as a feed in savory fraction separation via 0.2 µm membrane MF.

Effect of process condition of separation on CFMF performance.

Separation on autolysate via the 0.2 µm MF membrane at pump motor frequency of 10 Hz, room temperature and operation pressure of 2 and 4 bar for 0, 15, 20 and 25 minutes shows decrease of permeate flux value, as presented in Figure 3. Autolysate contains sufficient high dry matter (8.81 %), in which it is an accumulation of soluble and non-soluble components affecting on flow rate of solutes, so with pressure and time becoming more and more long will give different flow rate of solutes. Low pressure and long time of separation will result low flow rate of permeate via membrane. Higher pressure (4 bar) gives higher value of permeate flux than lower pressure (2 bar), because permeate passing through membrane becomes also higher, as a consequence of higher driving force.

Figure 3. Relationship between time and permeate flux value on separation of savory fraction from kidney bean *broth* autolysate using the 0.2 µm MF membrane.

The drop in the permeate flux value runs slow at initial process (0 – 15 minutes), because it does not occur yet accumulation of solutes (fouling) on the membrane surface to form gel layer (cake), so it occurs concentration polarization. Sharply decline of permeate flux value takes place after separation process for 20 minutes. This matter is possibility caused by its occurrence of a drag force, because the separation process is able to sweep solute particles on the membrane surface. Interaction between autolysate and membrane affects possibility on flow rate of feed and flow rate of permeate. At separation process of 25 minutes, permeate flux value becomes slower to constant condition or less permeate passing through membrane pores. At the end separation process (25 minutes), operation pressure of 4 bar gives higher value of permeate flux (42.7 L/m².hour) than 2 bar (30.83 L/m².hour).

Effect of process condition of CFMF on retentate and permeate compositions

N-Amino

Separation on autolysate of kidney bean *broth* through the 0.2 μm MF membrane at pump motor frequency of 10 Hz, room temperature and pressure of 2 and 4 bars with longer time will produce higher concentration of N-Amino in retentate than in permeate, as indicated in Figure 4. At the pressure of 4 bar for 5 and 10 minutes, N-Amino concentrations in permeate (8.4 and 9.8 mg/mL) were higher than in retentate (3.5 and 5.6 mg/mL), but after separation of 15, 20 and 25 minutes, N-Amino concentrations in permeate (4.2, 3.5 and 2.8 mg/mL) were lower than in retentate (7, 4.9 and 5.6 mg/mL). This case is possibility caused by its presence of N-Amino with particle size of 0.01 – 0.1 μm , so by using 0.2 μm MF membrane, this particle will pass freely. Besides different initial concentration, solubility property of N-Amino in water and interaction between components of autolysate and pH of autolysate are factors affecting its different recovery of separation product.

Figure 4. Effect of time on N-Amino in retentate and permeate as a result of separation of savory fraction from kidney bean broth autolysate through the 0.2 μm MF membrane.

At pressure of 2 bar, separation of N-amino is proportional and gives better product of separation, in which for separation of 5, 15, 20 and 25 minutes indicates higher concentration of N-amino in retentate (2.8, 6.3, 4.2, 4.2 and 4.9 mg/mL) than in permeate (4.2, 5.5, 3.5, 4.2 and 2.8 mg/mL). This case showed that higher driving force at pressure of 4 bar and initial separation (5 and 10 minutes) and N-amino are able to pass freely cross membrane as permeate product. After

15 minutes of process, on the membrane surface is possibility formed a cake layer, so that N-Amino particles interact with other components trapped in solid component, As a consequent, they are retained on the membrane surface. Lower driving force at operation pressure of 2 bar and larger particle size of N-Amino than 0.2 μm are retained on the membrane surface as retentate, compared with permeate. Based on process efficiency, at pressure of 4 bar and 15 minutes yields N-amino in retentate (7 mg/mL) as the highest savory fraction.

Dissolved Protein

Dissolved proteins have particle size range of 0.01 – 0.1 μm ⁽¹¹⁾, so that the 0.2 μm MF membrane will pass them through 0.2 μm MF membrane pores as permeate. Although, at higher pressure (4 bar) will be able to pass dissolved protein with smaller particle size than 0.2 μm in permeate. Whereas at lower pressure (2 bar), dissolved protein will be rejected in retentate. This case seems on higher dissolved protein concentration in retentate at pressure of 2 bar than 4 bar. In this condition, longer separation time will give higher dissolved protein concentration in retentate than in permeate, as showed in Figure 5. Increase of dissolved protein concentration in retentate occurred at pressure of 2 bar for 15 minutes (1.11 mg/mL) is optimal condition followed by fluctuated results. While, permeate shows an increase of dissolved protein concentration to final separation. Under pressure of 2 bar, separation gives better efficiency, as indicated by separating it successfully to the final process.

Figure 5. Effect of time on dissolved protein in retentate and permeate as a result of separation of savory fraction from kidney bean broth autolysate via the 0.2 μm MF membrane.

Total protein

Protein having particle size range of $0.04 - 2 \mu\text{m}$ and high molecular weight^(6,7) are polypeptides consist of a number of N-Amino. Protein of vegetable broth from kidney bean as a result of brine fermentation by *Rhizopus* sp-PL19 depends on type of initial proteinaceous material, namely phaseolin which is main globulin in kidney bean, is composed $\pm 50\%$ from total protein of kidney bean⁽¹⁴⁾ and 56% from total material in preparation of vegetable broth⁽¹⁵⁾. Brine fermentation by fungus/mold and autolysis processes cause its occurrence of fision of protein to peptides and N-Amino with lower molecular weight, although a part of polypeptides are hydrolyzed, so that at the separation through $0.2 \mu\text{m}$ MF membrane with different pressure and long time give retentate and permeate with different concentration of total protein.

CFMF technique at higher pressure (4 bar) is able to retain protein particle with shorter time to yield retentate with optimal protein concentration. This matter is reached at process of 10 minutes with total protein concentration of 12.09% (dry weight) because larger size of protein particles or similar of $0.2 \mu\text{m}$ are possibility present more much near and on the membrane surface than smaller size of protein particles of $0.2 \mu\text{m}$, so that larger size of protein particles than $0.2 \mu\text{m}$ will be retained on the membrane surface as retentate. This matter is possibility predicted that pump motor frequency (10 Hz) is proportional to pressure (4 bar) so that it is enough able to retain protein particles on the membrane surface. For the further process (15 – 25 minutes), protein particles passing in permeate become more and more much, and at the end process (25 minutes) is reached higher concentration of total protein in permeate (10.47%) than in retentate (7.11%), as given in Figure 6.

Figure 6. Effect of time on total protein in retentate and permeate as a result of separation of savory fraction from kidney bean broth autolysate via the $0.2 \mu\text{m}$ MF membrane.

Different trend seems for lower pressure (2 bar), in which CFMF technique will need longer processing time in order to get optimal total protein in retentate. This matter is represented for process of 20 minutes with optimal total protein in retentate (7.03%). This case is possibility caused that at pump motor frequency of 10 Hz and pressure of 2 bar, driving force of suspension feed used in CFMF technique is so high that protein amount passing through $0.2 \mu\text{m}$ MF membrane is higher (5 – 15 minutes), fluctuates in final separation, and shows higher concentration of total protein in permeate (7.85%) than that in retentate (5.51%). For all of the processes, CFMF technique at pressure of 4 bar gives better separation efficiency than 2 bar.

Fat

Fat has a particle size of $1 - 10 \mu\text{m}$, so that $0.2 \mu\text{m}$ MF membrane enables to retain and reject larger amount of fat in retentate than that passed as permeate. Although, other factors of process condition, such as type of material, flow rate, pressure and time, can affect on separation product. At pressure of 4 bar, CFMF technique gives successfully separation efficiency, and fat amount in retentate is higher than in permeate to 20 minutes of separation. While, for 25 minutes of separation gives higher fat amount in permeate, as given in Figure 7. Separation indicated that fat concentration in retentate becomes lower, and permeate relating with long time fluctuates. At pump motor frequency of 10 Hz and at particles are passing freely in permeate due to larger size of fat particle than $0.2 \mu\text{m}$. Different condition seemed in pressure of 2 bar, in which fat concentration in permeate reached is higher than in retentate to 20 minutes of process, and is higher in retentate (0.20%) than in permeate (0.13%) to final process (25 minutes). These whole process are possibility caused by smaller size of fat particles than $0.2 \mu\text{m}$ got in emulsification process via homogenization of 3,000 rpm, so that they passed in permeate and larger size of fat particle than $0.2 \mu\text{m}$ could only retained on the membrane surface.

Figure 7. Effect of time on fat in retentate and permeate as a result of separation of savory fraction from kidney bean broth autolysate through the $0.2 \mu\text{m}$ MF membrane.

Total Solid

Total solid present in autolysate cover all components contained in kidney bean, inoculum and rice substrate as a reaction result in brine fermentation and autolysis processes, such as colouring pigments and organic compounds resulted from thermal process of kidney bean *broth*. At pressure of 2 bar, CFMF technique is able to retain higher amount of total solids in retentate than in permeate to 15 minutes of separation time, but after 20 minutes of separation time, CFMF technique is not able to retain total solid particles and pass freely through membrane as permeate, so that at the final separation process (25 minutes) is obtained total solids concentration in retentate and permeate of 6.40 and 8.45 %, respectively, as showed in Figure 8. Different condition seems at pressure of 4 bar, in which total solids concentration in retentate (7.86 %) is higher than permeate (6.89 %) to the final separation time (25 minutes). For all of the processes, for 15 minutes of separation is critical time, in which difference in pressure can give a component change from optimal condition to less optimal condition. In other words, between 0 – 15 minutes separation time and pressure of 2 bar, total solids concentration in retentate is higher than at pressure of 4 bar, but between 15 – 25 minutes separation time and pressure of 4 bar, total solids concentration in retentate is higher than at pressure of 2 bar.

Figure 8. Effect of time on total solid in retentate and permeate as a result of separation of savory fraction from kidney bean broth autolysate through the 0.2 μ m MF membrane.

Salt

Salt is a component in autolysate conditioned so important that it affects on autolysis process. Salt content affects on autolysis system, especially recovery of savory fraction, such as protein, dissolved protein and

N-amino. Due to induction yield, it causes its occurrence *salting in* condition in order to increase savory fraction content⁽²⁾. In autolysate separation, salt content is arranged by suitable organoleptic in concentrate ranging from 0.5 to 0.8 % or less than 1 %⁽¹⁶⁾. Separation at pressure of 2 bar with long time results fluctuated concentration of salt in retentate and permeate to 20 minutes and becomes constant to final process (25 minutes), while at pressure of 4 bar gives constant concentration of salt to 25 minutes, as shown in Figure 9.

Figure 9. Effect of time on salt in retentate and permeate as a result of separation of savory fraction from kidney bean broth autolysate through the 0.2 μ m MF membrane.

Salt amount obtained in retentate is higher than in permeate. At pressure of 2 bar generates higher concentration of salt in retentate and permeate than 4 bar for all times because particle size of salt (0.001 – 0.01 μ m)^(6,7) is lower than membran pore size (0.2 μ m), so that at pressure of 4 bar, larger driving force of material than 2 bar will pass salt particles via membrane in permeate. Although, at pressure of 2 bar for 10 minutes of separation is obtained similar salt amount in permeate and retentate (1.66 %), fluctuation amount and at the end process (25 minutes) is reached higher concentration of salt in retentate (1.22 %) than in permeate (1.66 %). Different condition seems at pressure of 4 bar, in which salt concentration in retentate is higher than in permeate for all times and tends to be constant to 25 minutes of separation time with salt concentration in retentate of 1.06 % and in permeate of 1.01 %. For all of the processes, salt concentration in retentate as savory flavor is obtained optimally at pressure of 4 bar because it ranged lower (1.08 – 1.06 %) than 2 bar (1.40 – 1.22 %).

Reducing Sugar

Reducing sugar is monosaccharides having reduction property with smaller size of particle ($0.001\ \mu\text{m}$) than membrane pore size ($0.2\ \mu\text{m}$) so that it will pass freely across membrane as permeate, whereas sugar (monosaccharides, disaccharides) is generally retained on the membrane surface because of larger size of particle than $0.2\ \mu\text{m}$ ($8-20\ \mu\text{m}$). Although, operation condition factor (such as flow rate, temperature, pressure, time), sugar properties (particle size, solubility, interaction amongst components), and type of fouling can affect on sugar recovery in permeate and retentate⁽¹⁷⁾. Figure 10 represents the effect of operation condition on recovery of reducing sugar in permeate and retentate at pump motor frequency of 10 Hz, room temperature, and pressure of 2 and 4 bars. Reducing sugar in retentate and permeate seems a fluctuation change to 20 minutes of separation and to final separation (25 minutes). At pressure of 4 bar yields higher concentration of reducing sugar in retentate (71.75 mg/mL) than pressure of 2 bar (56.25 mg/mL). This condition showed that at pressure of 4 bar and 25 minutes start to be occurred fouling, so that sugar component will be trapped in cake layer and is retained on membrane surface, so sugar concentration in retentate will increase. While, at low pressure (2 bar), low driving force and smaller size of sugar particles than membrane pore size cause more amount of sugar particles passing in permeate than retained sugar particles in retentate. For all of the separations, $0.2\ \mu\text{m}$ MF membrane operating at pressure of 4 bar and 25 minutes indicated optimal efficiency of reducing sugar separation in retentate (71.75 mg/mL).

Hasil pengukuran daya antioksidan kuersetin dengan metode DPPH (Tabel 2) nilai $\text{IC}_{50} = 9,90\ \mu\text{g/mL}$. penangkapan radikal sebesar 50 %. Semakin kecil nilai IC_{50} berarti semakin kuat daya antioksidannya. Nilai IC_{50} sangat berarti karena menunjukkan kekuatan daya antioksidan. Bila kekuatan antioksidan kuersetin sebagai pembanding dibandingkan kekuatan antioksidan ekstrak n-heksana dan isolat murni *G. bancana* maka kekuatan antioksidan kuersetin > Isolat murni > ekstrak n-heksana ($\text{IC}_{50} = 9,90\ \text{g/mL} < 12,79\ \mu\text{g/mL} < 19,78\ \mu\text{g/mL}$). Dapat dikatakan bahwa kekuatan antioksidan ekstrak *n*-heksana *G. bancana* adalah sekitar 50 % dan isolat murni adalah sekitar 77 % dibandingkan dengan kekuatan pembanding kuersetin (disini bila dianggap kekuatan antioksidan kuersetin 100%)

Figure 10. Effect of time on reducing sugar in retentate and permeate as a result of separation of savory fraction from kidney bean broth autolysate via the $0.2\ \mu\text{m}$ MF membrane.

Figures of 11a and 11b showed a recovery of permeate and retentate through separation using CFMF technique at pump motor frequency of 10 Hz, room temperature and operating pressure of 4 bar for 10 minutes. From all separations of fermented kidney bean by *Rhizopus* sp.-PL19 through CFMF technique at pump motor frequency of 10 Hz, room temperature and pressure of 2 and 4 bars for 5, 15, 20 and 25 minutes were resulted a permeate, which is brownish yellow clear liquid and sharp savory flavor, while retentate is suspension with less sharp aroma.

Figure 11. Retentate (a) and permeate (b) as a result of savory fraction separation from fermented kidney bean autolysate through CFMF technique.

CONCLUSIONS

The experiment result showed that separation by means of CFMF technique gave permeate as savory fraction with better clearness level and sharper aroma than retentate, but retentate had better composition than permeate. During separation by means of CFMF technique increased total protein, dissolved protein and salt concentrations, but decreased total solid, reducing sugar and fat concentrations in permeate both pressure of 2 and 4 bar. Based on N-Amino concentration in permeate as source of savory fraction, the optimum condition of separation by means of CFMF technique was reached for 10 minutes both pressures of 2 and 4

bar. These conditions gave permeate flux value of 51.11 and 52.55 L/m².hour with concentrations of N-Amino of 5.50 and 9.80 mg/mL, dissolved protein of 1.01 and 0.97 mg/mL, total protein of 4.85 and 12.10 % (dry weight), reducing sugar of 55.75 and 53.75 mg/mL, salt of 1.16 and 1.06 %, and fat of 0.41 and 0.65 %, and total solids of 7.82 and 6.35 %, respectively. Retentate has a potential use as savory fraction concentrate for seasoning agent.

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