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Performance of Cross-flow Microfiltration in Separating Lactic Acid from Fermented Beetroot Biomass for Preventing Natural Oxidation

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----------ABSTRACT----------
Separation of lactic acid (LA) from fermented beetroot biomass was done as an effort to get permeate used as natural oxidation prevention and retentate. Separating was done by 0.15 µm microfiltration (MF) membrane equipped in plate-and-frame type cross-flow microfiltration (CFMF) membrane module and operated at room temperature, cross flow rate ~7.5 L/min and transmembrane pressure (TMP) 2 and 6 bar for 0, 5, 15, 25 and 35 minutes. The result of work showed that CFMF was able to separate the wanted component. Based on lactic acid recovery and process efficiency, optimization of process was obtained at room temperature, cross flow rate ~7.5 L/min and TMP 2 bar for 35 minutes and gave permeate flux of 8.05 L/m^2.h. with lactic acid 1.10%, total solids 3.41%, pH 3.12, and antioxidant activity 47.62%, meanwhile retentate contained lactic acid 1.66%, total solids 3.50%, pH 3.11 and antioxidant activity 62.45%. In this optimum condition, CFMF resulting retentate was able to raise lactic acid 74.74%, total solids 2.57% and inhibition 25.12% compared with starting separation process (0 minute). Permeate was dominated by monomer of lactic acid with molecular weight (MW) 91.153 Dalton (Da.) (M^{++}) and relative intensity 100%.
KEYWORDS: *cross-flow microfiltration, beetroot (Beta vulgaris L.), flux, lactic acid, inhibition.*

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I. INTRODUCTION

The beetroot (*Beta vulgaris* L) is a rich source of carbohydrate, protein, vitamins (B1, B2, B_6, B12, C), minerals (Mg, Fe, Na, K) and micronutrients [1]. Fermentation of beetroot by kombucha containing consortium culture of acetic acid bacteria, AAB (*Acetobacter* sp.), lactic acid bacteria (*Lactobacillus)*, yeast (*Saccharomyces cerevisiae*, *Saccharomycodes ludwigii*, *Zygosaccharomyces bailii)*, and kind of fungi (*Torolupsis* sp.) is conducted for 0 – 12 days [2]. Microbes in kombucha culture will hydrolyze sucrose to form ethanol and then oxidize to form acetaldehyde in order to form acetic acid and other organic acids (gluconic acid, glucuronic acid, lactic acid, malic acid) as antioxidant for anticholesterol. Organic acids contributes also to the desirable changes in fresh aroma like fruit aromas [3], [4]. Main role of organic acids impart a positive influence on the blood cholesterol levels caused by its ability in effecting both sterol and bile acid structure [5] through degradation of cholesterol to coprostanol so that number of cholesterol adsorbed by body is lower [6]. Property of lactic acid or 2-Hydroxypropanoic acid ($C_3H_6O_3$), is molecular weight (MW) 90.08 g/mol, acidity level (pK_a) 3.86, colorless to yellowish, soluble in water, ethanol, ether, and corrosive. One of the optical isomers forms of lactic acids, i.e. L(+) lactic acid is selected isomer implemented for food and pharmaceutical industries due to human body produces enzyme of L-lactate dehygronase [7]. Lactic acid is an one of the type of organic acids yielded through this fermentation and is well known as activated compound to prevent increase of cholesterol by stimulating LDL and trigliseride. Figure 1 displays chemical structure of lactic acid.

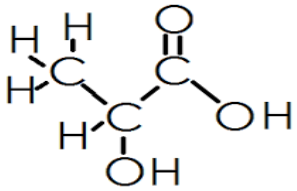


Fig. 1: Chemical structure of lactic acid.

In progress on fermented beetroot, microfiltration (MF) membrane by means of cross flow system is applied to separate lactic acid and other organic acids as natural antioxidant. In fermentation downstream processing, final products tend to be very dilute and contain a complex liquid which have molecular weight cut-off (MWCO) range approximately 500 – 2500, heat sensitive and degradable compounds. The optimization of a process is performed by selecting and adjusting of the membrane to the fermentation

product conditions [8], [9]. Generally, advances strategies to separate and purify fermented rootbeet have been based around MF techniques [10].

MF is one of the contemporary pressure-driven membrane processes which has become viable, non-thermal separation unit operations in chemical engineering. The word 'pressure-driven' means that the driving force is applied to achieve the desired hydrodynamic flow through the membrane and to separate micron-sized particles by a sieving and diffusion mechanism from a fluid into two liquids of different compositions. Pressure driven is governed by the hydrostatic pressure gradients (also known as trans-membrane pressure, TMP) across the selectively semi-permeable membrane. A feed introduced to a membrane separation system is separated into retentate (concentrate), the fraction that is physically sieved out on the surface of the membrane, meanwhile permeate, the fraction passing through the semi-permeable membrane. Cross-flow microfiltration (CFMF) is characterized by lower extent of concentration polarization and membrane fouling because of the crossflow or tangential movement of the feed when compared to dead-end microfiltration[11], [12]. MF membrane has pore sizes usually in the range of 0.1 – 10 μm, and are selectively designed to separate and remove micron-sized particles and other smaller solutes, and turbidity with linear dimensions ranging from 0.02 μm to 10 μm or particles with MWs in excess of 200,000 Dalton (Da.) from a solid/liquid suspensions. In its operation, MF involves the use of lower TMP, which is generally in the range of 1 – 6.2 bar [13], [14].

This research activity aims to investigate and evaluate implementation of MF membrane in separation of the desired compounds by measuring the permeate flux and the recoverability of total solids, total lactic acids, and antioxidant activity.

II. MATERIALS AND METHODS

II.1. Materials

The materials used in this study were fresh beetroot (*Beta vulgaris* L.) procured from local market, sucrose (local market). Kombucha culture (Research Center for Chemistry – LIPI) was used for hydrolyzing sucrose to form ethanol. Commercial fluoro polymer MF membrane (0.15 μm) (FSM-0.15-PP, Alfa Laval, Nakskov, Denmark) with membrane area 0.072 m^2 (2 sheets of membranes) was utilised to separate and/or purify the desired compounds from fermented beetroot. All chemicals applied in analyzing total solids, total acids (lactic acid) and testing antioxidant activity were obtained from commercial sources in local market and analytical grade quality.

II.2. Equipment

The tools used in this study were a series of fermentation system in semi-pilot (local), plate-and-frame type cross-flow membrane filtration module with both adjustable membrane area and operational pressure (DSS LabUnit M20, Danish Separation Systems AS, Denmark) and analysis instrument consisting of UV-Vis Spectrophotometer (Model RF-550, Shimadzu, Japan), Liquid Chromatography tandem Mass Spectrometry (Mariner Biospectrometry) installed with LC (Hitachi L 6200) and Particle Size Analyzer (PSA) with SZ 100-nano Partica Dynamiv Light Scattering (DLS) system (Beckman Coulter LS 100 Q, U. S. A) [15].

II.3. Procedure

II.3.1. Experimental design

This study was conducted by using biomass of beetroot fermented by kombucha culture in semi-pilot scale (7 L). This biomass was separated the wanted compounds by using MF membrane (0.15 μm) installed in CFMF at a fixed condition, i.e. room temperature, pump motor frequency 20 Hz (cross flow rate ~7.50 L/min.), and TMP 2 and 6 bar for 0, 5, 15, 25, and 35 minutes. Analyses were carried out on original (initial) biomass of fermented beetroot (feed), permeate and retentate consisting of total solids (Gravimetric method), lactic acid (Titratable method) [16] and ability of inhibition [17]. Performance on MF membrane (0.15 μm) equipped in CFMF module was done on flux and recoverability [18]. Sample from the best treatment condition was conducted identification of lactic acid by LC-MS [15]. Process and chemical analysis were done with an average of at least two measurements. Data processed in this description were based on the result of average analysis.

II.3.2. Fermentation process of beetroot in semi-pilot scale

A number of beetroot was subsequently carried out by reducing size, blanching at 80 ºC for 5 minutes, pulverizing with sterilized water in a 1 to 4 ratio, and sieving via a 60 mesh sieve until filtrate and residue were yielded. Filtrate was pasteurized at 90 – 95 °C, cooled and added with sucrose 10% (w/v filtrate), inoculated by maintenance kombucha culture 10% (v/v filtrate), and stored in closed container for 7 days so that inoculum of fermented beetroot was resulted. Process of fermentation in semi-pilot scale (7 L) was employed with similar starting step. Beetroot was pulverized in a 1 part : 8 parts mixture with sterilized water, sucrose concentration 10% (w/v filtrate) and beetroot inoculum concentration 10% (v/v filtrate). Fermentation was conducted in closed container at room temperature for 30 days until

fermented broth stated as fermented beetroot biomass was yielded. Fermented beetroot biomass was sieved through a 120 mesh sieve so that filtrate of fermented beetroot biomass and residue was generated. Filtrate of this fermented beetroot biomass was used as feed in separating and/or purifying the desired compounds by means of CFMF technique.

II.3.3. Separation of the desired components from fermented beetroot biomass via MF membrane

The flat sheet MF membrane configurations have a 0.15 μm pore size with the smooth membrane side upwards and the rough membrane side downwards facing the plate was installed in the CFMF module. Before use, membrane installed in CFMF module (9 L) was rinsed using pure water. Membrane being installed was compacted by flowing pure-water to the membranes at TMP 2 bar for 5 minutes. Compaction ensured that all remainded solvents used during the manufacturing were removed from the surface of membrane and pores. Pure water in the feed tank of module was drained and filled with 3.5 L of fermented beetroot biomass. The feed of fermented beetroot biomass in the feed tank is pumped at cross flow rate of ~7.5 L/min. through a pre-filtered in 200 μm filter, heat exchanger, and into a membrane module (plate & frame) mounted vertically at the average pressure of inlet and outlet pressure of 6 bar until the permeate passing via membrane surface area was measured volume with a period of 0, 5, 15, 25, and 35 minutes, respectively so that permeate flux could be calculated. While, the rejected component in feed, which do not passes through the membrane, is re-circulated continuously to the feed solution tank as retentate. With the similar procedure and condition, separation of fermented beetroot biomass was done through membrane of MF at TMP 2 bar. The wanted components in permeate and retentate were analyzed. After use, membrane installed in CFMF module was subsequently flush and rinsed with pure water, NaOH solution, and pure water to achieve pH 8.5 – 10.5.

II.3.4. Analysis of antioxidant activity

Antioxidant activity from extract on free radical of DPPH was carried out on aliquot of retentate and permeate as the best result of CFMF operation (TMP 2 bar, 35 minutes). A number of retentate or permeate was subsequent extracted with Ethyl Acetate, dried at 40 ºC, added aquadest to concentration 100 ppm (0.2 mg in 2 mL of aquadest), and added 0.5 mL of DPPH (1 mM in methanol). Mixture was shaked and allowed to room temperature for 30 minutes. Absorbance yielded was monitored at the wavelength of 515 nm. Inhibition (%) was calculated based on difference in adsorption between blank and sample. Calculation was performed based on effect of absorbance of DPPH, as follows : Effect of absorbance of DPPH-scavenging (%) = $[1 - (A_s/A_o)\times100]$, in which A_o, Absorbant of blank and A_s, Absorbant of sample. Percentage of activity of DPPH absorbance was plotted on concentration of sample. Absorbance value 50% (IC_{50}) was calculated according to graphic of absorbance percentage on concentration of sample [17].

III. RESULTS AND DISCUSSION

II1. 1. Characteristics of material

Fermentation of beetroot by kombucha culture was performed by using a semi-pilot scale fermentor (7 L) in closed container at room temperature for 30 days. During fermentation run, change in physical property and chemical composition takes place, in which biomass colour changes to lighter colour and fresh aroma. This case occur due to effect of microbes in hidrolyzing sucrose and beetroot sugar to organic acids, such as lactic acid. Composition of filtrate of fermented beetroot passing via a 120 mesh sieve showed concentrations of total solids 3.5024% and total acids 1.65 %, pH 3.14, and antioxidant activity 63.01%. This composition is different in initial pulverized beetroot (pulp), fermented beetroot biomass for 12 days, and fermented beetroot biomass for 30 days, as shown in **Table 1.**

Table 1. Composition of pulp of beetroot, and both fermented beetroot biomasses for 12 days and 30 days.

Components	Kind of materials		
	Pulp of beetroot	Fermented beetroot biomass 12 days	Fermented beetroot biomass 30 days
Total acids (%)	0.06	0.50	1.65
Total solids (%)	10.22	10.07	3.50
pH	6.38	3.64	3.14
Inhibition (%)	85.59	91.74	63.01

Composition of initial pulp of beetroot yielded in laboratory scale fermentation (1 L) gave concentration of total lactic acid 0.06% and total solids 10.22%, pH 6.38, and antioxidant activity as inhibition 85.59% [19]. Meanwhile, composition of fermented beetroot biomass yielded via laboratory scale fermentation (1 L) for 12 and 30 days indicated concentrations of total lactic acid 0.50%

and 1.65, total solids 10.07% and 3.50 %, pH 3.64 and 3.14, and antioxidant activity as inhibition 91.74% and 63.01%, respectively. Fermentation process caused both change in physical property and chemical composition of suspension because of hydrolysis and environmental factors (temperature, time, concentration of beetroot inoculum). Fermentation time becoming more and more long increases pH, total acids and inhibiting ability, but decreases total solids at beetroot without fermentation to 12 days of fermentation in one (1) L volume. This increase of ability of inhibition is possibility caused by occurrence a conversion cholesterol to coprostanol [6] as a consequence of increase of total acids. Nevertheless, this ability of inhibition decreases in larger scale fermentation (7 L) to 63.01%, although total acids concentration experiences an increase to 230% (2.3-folds) in comparison to 12 days of fermentation from 0.5% to 1.65% at 1 L of fermentation volume (1.65%). This matter takes place by autolysis dropping inhibition ability of biomass. Figure 2 demonstrates pulp of beetroot, fermented beetroot biomass (12 days), and fermented beetroot biomass (30 days).

Fig. 2: (a) pulp of beetroot, (b) fermented beetroot biomass for 12 days, and (c) fermented beetroot biomass for 30 days.

III.2. Influence of process condition of MF membrane on fluxes

Criteria of ideal membrane performance consist of *performance* (flux and selectivity) and resistance. The flux is usually the quantity of permeate volume (L) passing through unit area of membrane per unit time [20]. The flux is influenced by many factors, such as feed concentration, TMP, cross-flow velocity, temperature, and time. High feed concentration causes a decline of flux until a near equilibrium filtration rate [21]. In separation of organic acids as lactic acid at room temperature, pump motor frequence 20 Hz (cross flow rate of ~7.5 L/min.), and TMP 2 and 6 bar for 0, 5, 15, 25 and 35 minutes displays time becoming more and more length will lessen flux at both TMP, in which the differences in the permeate flux at TMP of 6 bar was higher than that at TMP of 2 bar for all times due to higher driven force, as presented in Figure 3.

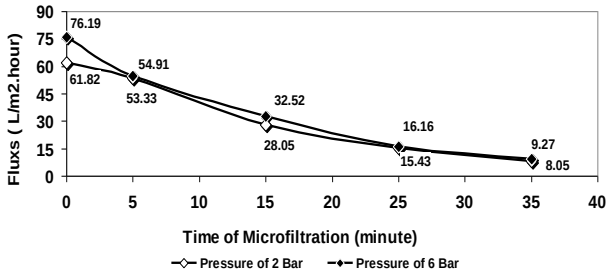


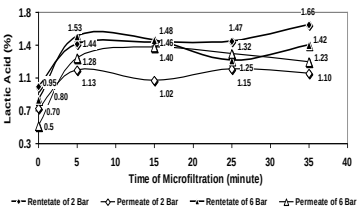
Fig. 3: Variation of permeate flux with time of MF at TMP 2 and 6 bar (room temperature, cross flow rate ~7.5 L/min.)

At both TMPs, the permeate fluxes are tend to drop sharply to 25 minutes of process followed by declining gradually to 35 minutes. Fluxes yielded at TMP 2 bar for 25 minutes and 35 minutes are 15.43 L/m^2.h. and 16.16 L/m^2.h., respectively. Meanwhile, the permeate fluxes resulted at TMP 6 bar for 25 minutes and 35 minutes are 8.05 L/m^2.h. and 9.27 L/m^2.h., respectively. The differences in the permeate flux at TMP of 6 bar was higher than that at TMP of 2 bar for all times. On MF membrane (0.15 µm), the increase in TMP from 2 to 6 bar increased the initial permeate flux by 15.5% and increased the average flux over a period of 35 minutes by 11.9%. Declining the permeate flux during membrane process is mainly caused by fouling, in which the occurrence of the accumulation of component particles (such as total solids, total acids, etc.) in the membrane pores changing the effective membrane pore size distribution and the membrane surface forming a deposit layer [22]. Under the conditions of constant TMP and cross-flow velocity (flow rate), permeate flux in CFMF drops to a steady-state value which can be lower than the initial permeate flux so that TMP do not affect on flux for the whole process because fouling start occur. The occurrence of membrane fouling reduces microfiltration performance (flux and recoverability), increases the total hydraulic resistance, and increases energy consumption. This

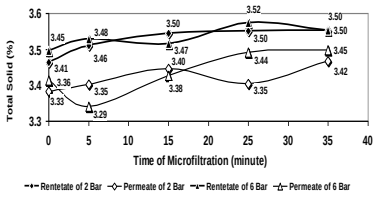
case shows relationship between performance of membrane and components of material, particularly organic acids stated as lactic acid is considered optimum.

III.3. Influence of process condition of MF membrane on composition

Lactic acid is one of the organic acids produced through degrading carbohydrate by kombucha culture. Organic acids have smaller particle size (0.001 – 0.01 µm) or molecular weight (MW) in the ranges of 60 to 90 Dalton (Da.) in comparison to pores of MF membrane (0.15 µm) [23] so that much more particles should pass across membrane than that them retained in retentate. At TMP 2 bar, CFMC system separates lactic acid successfully due to much more lactic acid particles retained in retentate in comparison to them passing across permeat for all process times. On the other hand, at TMP 6 bar, CFMF separates lactic acid unsuccessfully because of much more lactic acid penetrating across permeate in line with long process time, as shown in Figure 4a**.** This case is caused by higher TMP (6 bar) to force liquid fluid through membrane so that to retain lactic acid particles on membrane surface is required shorter process time (5 minutes). For longer process time, this large driven force is able to sweep component of lactic acid so that much more lactic acid particles passes through membrane as permeate. For process at TMP 2 bar, driven force is insufficient high to force liquid fluid across membrane so that fouling takes place sufficient long (35 minutes). As a consequence, system will concentrate lactic acid particles on the top membrane surface. The best optimization of lactic acid is achieved at TMP 2 bar for 35 minutes. In this condition, CFMF system is able to retain lactic acid in retentate 74.74%, and passing in permeate 57.14% in comparison to initial process (0 minute), namely from 0.95% to 1.66% and from 0.7% to 1.1%, respectively after MF process at TMP 2 bar for 35 minutes. On total solids, CFMC system separate total solids successfully for both TMPs because much more total solid particles are retained on retentate than that passing via membrane as permeate, in which on TMP 6 bar gives higher total solids than that TMP 2 bar for all proces times, as demonstrated in Figure 4b. At higher TMP (6 bar) needs shorter process time (25 minutes) in comparison to lower TMP (2 bar) requiring longer process time (35 minutes) in order to get optimum concentration of total solids, namely 3.52% and 1.50%**.** Difference in optimization is not only caused by difference in driven force, but also by CFMF with treatment at range of short time and kind of material like colloid so that process run fast, which it does not give a chance polarization of total solids on the top membrane surface. Total solids are all of total soluble and total insoluble from the entire components in feed. Accumulation of all components during process is enabled to form aggregate with larger particle size in comparison to pores of MF membrane (0.15 um) by influence of membrane system (TMP, cross-flow velocity, time, type of membrane, and kind of material) [24]. Optimization of recovery of the best total solids (3.52%) is achieved at TMP 6 bar for 25 meinutes. In this condition, CFMC system is able to retain total solids (2.24%) in retentate and pass to permeate (2.30%) in comparison to initial process (0 minute) from 3.45% to 3.52% and 3.36% to 3.44%. On pH value, CFMF system gives acidity level becoming more and more drop in retentate and permeate, however, it increases acidity level in permeate in line with the length of process time at both TMPs for all process times, as presented in Figure 4c. This case is possibility relating to compositions of organic acids yielded during fermentation process (acetic acid, lactic acid, butiric acid, propionic acid, etc.) contributing on acidity level. At TMP 2 bar, acidity level in permeate is higher than that acidity level in rentetate because much more organic acids passes freely in permeate for all process times. Particle size of organic acids (0.001 – 0.1 µm) are smaller than that pores size of MF membrane (0.15 µm) [24]. At higher TMP (6 bar) is enabled occurrence of fouling so that much more organic acid particles are accumulated on the top membrane surface in comparison to passing in permeate causing increase of acidity level or decline of pH value. The best process condition of acidity level was achieved at TMP 6 bar for 35 minutes giving acidity level in retentate 3.10 and in permeate 3.13. In this optimum process condition, CFMF system is able to raise acidity level in retentate 1.90% in comparison to acidity level at initial process (0 minute) (3.16%).



(a)



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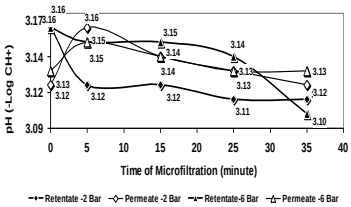

(c)

Fig. 4: Relationship between time of MF and TMP on (a) lactic acid, (b) total solids, and (c) pH in retentate and permeate of fermented beetroot biomass by CFMF.

III.4. Optimum process condition

Based on process efficiency on lactic acid, separation process of desired and target components from fermented beetroot was achieved at room temperature, flow rate ~ 7.5 L/min. and TMP 2 bar for 35 minutes giving flux value 8.05 $L/m^2.h$. In this condition was yielded retentate with lactic acid concentration 1.66%, total solids concentration 3.50%, and pH 3.11, whereas permeate gave lactic acid concentration 1.10%, total solids concentration 3.41%, and pH 3.12. In this optimum condition, CFMF system is able to increase total lactic 74.74% and total solids 2.57% in retentate and decrease total lactic concentration 57.14% and total solids concentration 2.50% in permeate in comparison to concentrations of total lactic and total solids in retentate and permeate for starting separation time (0 minute). Figure 5 presented retentate and permeate of fermented beetroot as a result of CFMF system at room temperature, flow rate ~ 7.5 L/min., and TMP 2 bar for 35 minutes.

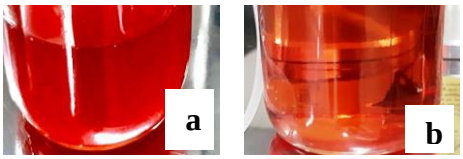

Fig. 5: (a) Retentate and (b) permeate of fermented beetroot biomass resulted from CFMF at room temperature, cross flow rate ~ 7.5 L/min. and TMP 2 bar.

III.5. Antioxidant activity (inhibition)

Based on recovery of lactic acid, both activities of antioxidant on retentate and permeate from the best treatment (room temperature, crossflow rate ~7.5 L/min., TMP 2 bar, 35 minutes) displaying ability of inhibition 62.45% and 47.62%, respectively are higher than that on retentate (56.48%) and lower than that permeate (52.43%) at same process condition but at TMP 6 bar, as demonstrated in Figure 6. This case is possibility caused by presence of relationship between lactic acid concentrations for both TMPs for same process time (35 minutes). At TMP 2 bar, lactic acid concentrations in retentate 1.66% and permeate 1.10% are higher than those in retentate 1.42% and permeate 1.23% at TMP 6 bar. For the entire processes, CFMC system at TMP 2 bar for 35 minutes is able to increase inhibition at retentate 25.12%, however decreases inhibition at permeate 4.59% in comparison to starting process (0 minute), 49.91%. At TMP 6 bar for 35 minutes, CFMC system is able to increase inhibition at retentate 11.25% and permeate 3.27% in comparison to initial process (0 minutes), 50.77%. This case indicated that at high TMP (6 bar), at permeate has better ability of antioxidant but insufficient ability of antioxidant presents at retentate in comparison to process at lower TMP (2 bar).

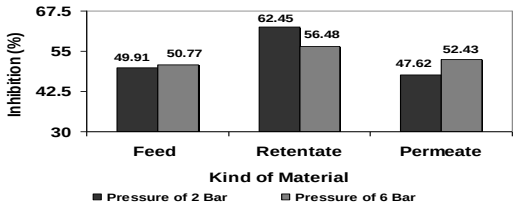

Fig. 6: Relationship between kind of material and TMP on antioxidant activity (inhibition) from fermented beetroot yielded from CFMF at room temperature and cross flow rate ~7.5 L/min. for 35 minutes.

III.6. Identification on lactic acid monomer in fermented beetroot biomass

Identification on lactic acid was performed using a LC-MS. Electrospray ionization (ESI) mass spectrometry was conducted in both the positive and negative ionization mode. Lactic acid has molecular weight 90.8 Da. By using LC-MS method had been known that a compound indicated difference in MW, in which its possibility is as M^+, M^+ Na^+, $2M^{++}$ or $2M^+$, Na^+ [15]. The operation conditions were the injection volume 5 µL, the flow rate 0.2 mL/min., methanol as eluent, and a C-8 column (15 mm x 2 mm). At standard lactic acid was get chromatogram at one (1) peak (T1.3), retention time 0 – 10 minutes and relative intensity 100%, as seen in Figure 7a. Mass spectra was

checked over the *m/z* range of 91.00 – 92.20. T1.3 displayed domination of monomer with MW 91.61 Da. (M^+) with relative intensity 100%, as showed in Figure 7b. Identification on lactic acid was performed on permeate of *fermented beetroot* from the best treatment based on total acids at room temperature, crossflow rate ~7.5 L/min. and TMP 2 bar for 35 minutes. Chomatogram on permeate was dominated by peak T1.7 at retention time 0 – 10 minute, as shown in Figure 7c. Mass spectra checked over the *m/z* range of 91.0 – 92.0 was yielded 10 monomers of lactic acid with MW ranging between 91.06 Da. and 91.98 Da. Dominated by one (1) monomer of lactic acid with MW 91.153 Da. (M^+) with relative intensity 100%, as showed in Figure 7d.

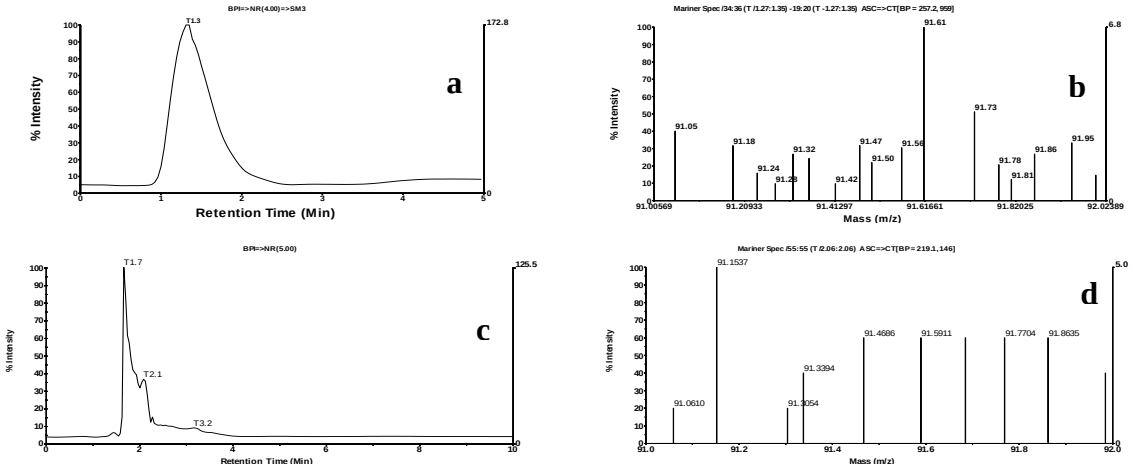

Fig. 7: (a) Chromatogram of standard lactic acid, (b) mass spectra of standard lactic acid, (c) chromatogram of fermented beetroot (permeate), and (d) mass spectra of fermented beetroot (permeate) with optimum condition of CFMF at room temperature, flow rate ~ 7.5 L/min. and TMP 2 bar for 35 minutes.

IV. CONCLUSION

CFMF technique with different TMP and separation time gave successfully separation on lactic acid and total solids, however in separating fermented beetroot biomass in moduke scale takes place a drop of flux. Separation time becoming more and more long at both TMPs will raise lactic acid and total solids concentrations, however declines pH value in retentate. In lower TMP (2 bar) will need longer separation time than that higher TMP (6 bar) in order to achieve the optimum lactic acid concentration. Based on lactic acid recovery and process efficiency, optimization of process condition was reached at room temperature, crossflow rate ~7.5 L/min. and TMP 2 bar for 35 minutes and gave permeate flux of 8.05 $L/m^2.h$. having concentrations of lactic acid 1.10% and total solids 3.41%, pH 3.12, and antioxidant activity 47.62%, whereas retentate had concentrations of lactic acid 1.66% and total solids 3.50%, pH 3.11, and antioxidant activity 62.45%. In this optimum process condition, CFMF technique yielding retentate was able to increase concentrations of lactic acid 74.74% and total solids 2.57%, and antioxidant activity 25.12%, but in permeate takes place a decrease on concentrations of total lactic 57.14% and total solids 2.50%, and antioxidant activity 4.59% in comparison to concentrations of total lactic, total solids and antioxidant activity in both retentate and permeate at initial time of separation (0 minute). Permeat was dominated by monomer of lactic acid with MW 91.153 Da. (M^{++}) and relative intensity 100%.

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