Difference in Characteristic of Beetroot (Beta Vulgaris L.) Biomass through Microfiltration Technique to Prevent Natural Infection

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Keywords: Beetroot (*Beta Vulgaris L.*), Betacyanin, Polyphenol, Microfiltration (MF), Antibacteria.

Abstract: Fermentation of beetroot (Beta vulgaris L.) by using Kombucha culture produces biomass as a source of polyphenol, particularly betacyanins having potential use as prevention of natural infection. This experiment activity aims to find out separation optimization of beetroot biomass through microfiltration (MF) technique and characteristic of retentate and permeate as a result of MF on composition, monomer domination of polyphenol and betacyanin compounds, particle size distribution, and ability of anti-bacteria to prevent natural infection on *Staphylococcus aureus* Ina CC-B4 and *Escherichia coli* Ina CC-B5. Separation was conducted at room temperature, strirrer rotation speed (SRS) 200, 300 and 400 rpm, and fixed transmembrane pressure (TMP) 40 psia. Result of experiment work showed that based on betacyanin, the best treatment is achieved at SRS 300 rpm yielding retentate and permeate with composition of betacyanin of 0.31 and 0.16 µg/mL, total polyphenol of 0.55 and 0.37%, total acids 1.00 and 0.72%, reducing sugars 16.33 and 28.97 mg/mL, total solids 10.05 and 9.38%, dissolved protein 18.50 and 24.35 mg/mL, particle size 3002.4 and 1962.0 nm, particle index 0.468 and 0.370, respectively. Identificaion on betacyanin and gallic acid monomers as total polyphenol at retentate is dominated by monomer with molecular weight (MW) 551.13, 551.53, and 171.02, 171.24 and 171.76 Dalton (Da.), meanwhile permeate is dominated by monomer with MW 551.16 and 171.23, 171.72 Da. and relative intensities 100%, respectively. Ability to inhibit the growth of bacteria of *Staphylococcus aureus* Ina CC-B4 and *Escherichia coli* Ina CC-B5 is achieved by retentate with zone area of inhibiting 11 and 10 mm, respectively. In this optimum condition, MF membrane technique was able to retain betacyanin and total polyphenol as anti-bacteria compounds in retentate with 416% (4.16-folds) and 83.33% and pass them in permeate 166% (1.67-folds) and 23.33% compared to prior to process (feed).

1 INTRODUCTION

Fermentation of beetroot (*Beta vulgaris L.*) by Kombucha cultures generates biomass with beneficial as source of polyphenol and natural organic acids as functional food. Beetroot is the deep violet tubers by betacyanin pigment (Sarkar *et al*, 2015). being having potential uses as antioxidant, anti-cholesterol, natural detoxification, and prevention of hypertension. Betacyanin including in betalain pigment can be categorized as phenolic compound (Coultate, 2009). Betalain pigment is synthesized from amino acid of tirosyne containing nitrogen element (Pavoković & Krsnik-Rasol, 2011) as shown in Fig. 1. Betacyanin has a property of exclusive-mutual on anthocyanin pigment. In other words, both these pigments are not found together with anthocyanin (Cai *et al*, 2005; Grotewold, 2009).

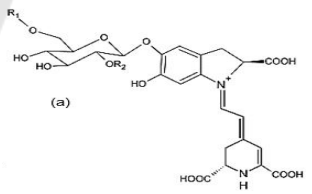

Figure 1: Chemical structure of betacyanin.

Recovery of betacyanin as biomass of fermentation by Kombucha culture is related to microbe activity in degrading sucrose into glucuronic acid, acetic acid, malic acid (Malbaša *et al*, 2008) and other compounds, such as betacyanin being possibility has beneficial to prevent infection of *Stapylococcus aureus* Ina CC-B4 and *Escherichia coli* Ina CC-B5 through chemical reactions assimilative and dissimulative during fermentation (0 –12 days). In its function as natural detoxification, they have the

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ability to prevent infection-related to the role of betacyanin as phenolic compounds.

Betacyanin in retentate (concentrate) and permeate as a result of MF on fermented beetroot suspensions have potential use as prevention of natural infection being generated via separation by means of MF membrane. MF membrane is able to sieve particles with particles in the ranges larger than 0.1 to 10 μm at trans membrane pressure (TMP) 5 – 50 psia (0.3 – 3.3 bar), so that it retains fat particle (1 – 10 μm), protein (0.04 – 2 μm), polysaccharides (8 – 20 μm) on the membrane surface. Particles with size in the ranges of smaller than 0.1 to 10 μm will pass freely as permeate, particularly colour pigment, such as anthocyanin, chlorophyll, β-carotene (0,001 – 0.1 μm), organic acids, amino acids, vitamin and mineral (0.001 – 0.1 μm) (Raja *et al* 2003; Field *et al* 1993 and Mulder, 2012). The presence of *fouling* becomes a possibility when compounds retained on the membrane surface are affected by type of membrane, characteristic of biomass, operation condition (temperature, flow rate or stirrer rotation speed, trans membrane pressure, TMP) (Field, 1993; Mulder 2012). MF membrane being made of fluoropolymer has specification with pores size in the ranges of 0.15 μm to 0.65 μm and is able to operate at TMP in the range of 1 bar to 10 bar (module scale) or TMP in the ranges of 20 psia to 40 psia (dead-end stirred ultrafiltration cell, DESUFC), flow rate in the ranges of 3.5 L/minute to 15 L/minute (module scale) or stirrer rotation speed (SRS) in the ranges of 200 rpm to 400 rpm (DESUFC), temperature in the ranges of 0 to 60 °C and pH ranges of 1 to 11 (Millipore, 2008), so that assessment on the effect of SRS at fixed TMP enables to get optimization of betacyanin. Characterization of betacyanin compounds in fermented beetroot biomass is performed by identifying betacyanin compounds using Liquid Chromatography coupled with Mass Spectrometry (LC-MS). By using LC-MS, MWs range of purified polyphenol compound could be known and estimated so that the functional property of betacyanin could be declared. Chromatography separates mixtures of molecular based on difference in migration speed and molecules distribution in stagnant phase (adsorbent) and moved phase (eluent), while mass spectrometry ionizes analytes based on the principle of electrospray ionization (ESI) to the gas phase (fine aerosol) (Onggo et al, 2009). LC-MS will separate betacyanin monomer and identify MW (Eichhorn, 2001), whereas particle size and particle size distribution can be known by means of Particle Size Analyzer (PSA) (Dapkunas *et al*, 2001); Retsch-Technology GmbH, 2019). In progress, the ability of anti-bacteria activity of *Staphylococcus aureus* Ina CC-B4 and *Escherichia coli* Ina CC-B5 in permeate and concentrate as a result of separation of target and desired compounds from fermented beetroot biomass through MF membrane was performed via analysis of microbiology covering inhibition power to the growth of both types of microbes. *Staphylococcus aureus* is a Gram-positive, a facultative anaerobe, round-shaped bacterium with diameter size (Ø) ranging 0.8 – 1.0 μm, and the optimum growth at 37 ºC. *S. aureus* can become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections associated with various pathologies condition, such as sinusitis, pneumonia, meningitis, arthritis, and food poisoning (Madigan et al, 2008). Meanwhile, *Escherichia coli* known *E. coli* is a non-spore-forming, Gram-negative, facultative anaerobic, rod-shaped, and coliform bacterium of the genus *Escherichia which is* harmless, but some serotypes can cause serious food poisoning and contamination in their hosts because it produces exotoxin, which can stop synthesis of protein (Levinson, 2008). Characteristic of biomass, concentrate, and permeate on the domination of betacyanin monomer, particle size, and particle size distribution are enabled to affect on ability of their materials in inhibiting the growth of *Staphylococcus aureus* Ina CC-B4 and *Escherichia coli* Ina CC-B5. This matter enables to get the functional property as anti-bacteria being can prevent infections of both microbes.

The aim of this experiment work was to find out process optimization of MF based on difference in SRS on composition, particularly total polyphenol and betacyanin, monomer domination of polyphenol and betacyanin, particle size, particle size distribution, and ability to inhibit the growth of bacteria of *Staphylococcos aureus* Ina CC-B4 and *Escherichia coli* Ina CC-B5.

2 MATERIALS AND METHODS

2.1 Materials and Equipments

The materials used for this experiment activity were fresh beetroot tuber procured from local market, Kombucha culture (Research Center for Chemistry – LIPI), sucrose, *Staphylococcus aureus* Ina CC–B4 and *Eschericiae coli* Ina CC-B5 bacteria (Research Center for Biology – LIPI), commercial composite fluoropolymer MF membrane with pore size of 0.15 μm (FSM-0.15-PP, Alfa Laval, Nakskov, Denmark) (according to the manufacturer), and chemicals with

analytical grade quality used for preparation and analysis purposes, such as gallic acid.

Meanwhile, main equipments applied in this experimental activity were digital balance (Fujitsu, Japan), peeler (local product), autoclave (Cheng Yi, LS-50 L, China), blender (National, local), sieve of 60 mesh (Retsch, Germany), incubator (local), series of fermentation system in laboratory scale (local), stopwatch (Hanhart Profil2, Germany), magnetic stirrer (HI 303 N, HANNA Instrument, Japan), pressure gauge of technical nitrogen (Fisher Scientific Company, England), cylinder tank for technical nitrogen (local), Dead-End Stirred Ultrafiltration Cell (DESFC) (MILLIPORE Model 8200, U.S.A.), UV-vis Spectrophotometer (Model RF-550, Shimadzu, Japan), Liquid Chromatography-tandem Mass Spectrometry (Mariner Biospectrometry) equipped with LC (Hitachi L 6200) [2] and Particle Size Analyzer (PSA) with SZ 100-nano Partica Dynamic Light Scattering (DLS) system (Beckman Coulter LS 100 Q, U. S. A) (Dapkunas *et al*, 2001).

2.2 Experimental Design

Experiment activity was conducted using beetroot biomass fermented by Kombucha culture. Beetroot biomass was concentrated through a MF membrane fitted in DESUFC at room temperature, SRS 200, 300 and 400 rpm, and TMP 40 psia for 30 minutes. Analysis was conducted on initial material of biomass (feed), retentate, and permeate covers total solids (Gravimetric method) (AOAC, 2019), total acids (titratable acids method), total polyphenol (Folin-Denis or Folin-Ciocalteu method) (Liu, 2006), dissolved protein (Lowry method)(Lowry, 1951), and betacyanin (Wong *et al*, 2015). Aliquot from the best condition treatment was performed by identifying betacyanin by means of LC-MS (Eichhorn, 2001) and anti bacteria activity of *Staphylococcos aureus* Ina CC-B4 and *Escherichia coli* Ina CC-B5 through investigation of inhibition zone (Bell et al, 1984), particle size distribution through PSA (Dapkunas et al, 2001). Process and analysis were performed in duplicate. Data were processed in this description based on result of average analysis.

2.3 Procedure

A series of the process initiated by preparing inoculum of fermented beetroot covers sorting, cutting of beetroot, blanching at 80 ºC for 5 minutes, pulverizing by adding clean water at ratio ranged from 1 to 4, and filtering via 60 mesh in order to get filtrate. Further, filtrate was autoclaved at 90 – 95 °C, added sucrose 10% (w/v, filtrate), cooled, inoculated with Kombucha culture 10% (v/v, filtrate), and fermented in closed container in darkroom so that it is produced inoculum of fermented beetroot. Fermentation process was conducted with similar initial step, however process of pulverizing beetroot was carried out by adding clean water at ratio of beetroot/water (1 : 8, w/w), sucrose 10% (w/v filtrate of beetroot) and inoculum of fermented beetroot 10% (v/v filtrate of beetroot), and fermentation of mixture of pulverized beetroot, sucrose and inoculum of fermented beetroot was performed in closed container in darkroom at room temperature for 12 hours so that it is generated biomass of fermented beetroot. This recovery of biomass of fermented beetroot is used as feed in purifying by means of MF membrane. Separating and/or concentrating biomass of fermented beetroot was conducted through fitted in DESMFC mode with a capacity of 180 mL (laboratory scale). Empty DESMFC was filled by biomass of fermented beetroot, flown by nitrogen gas at a pressure of 40 psia, and stirred at room temperature, SRS of 200 rpm and TMP of 40 psia for ± 0 (initial process) and 30 minutes (Millipore. 2008). Both permeate passing across MF membrane and remained retentate were collected in small beaker glasses and analysed. After used, the membrane was washed with distilled water. This procedure is conducted according to experimental design.

3 RESULTS AND DISCUSSION

3.1 Characteristic of Materials

Composition of beetroot before and after fermentation process was tabulated in Table 1, in which it had been appeared that fermentation process generates biomass with composition of dominant on polyphenol in its function as prevention of natural infections. The fermentation process increases organic acids and reducing sugars, but decreases almost the whole components. Increasing both these components are caused by activity of Kombucha culture degrading sucrose and produce ethanol followed by oxidizing ethanol to acetaldehyde and then generate acetic acid. Accumulation from each metabolite will form organic acids, such as glucorunic acid, acetic acid, malic acid, butyric acid, and other components as antioxidant. Betacyanin is able to bind sugars so that total acids is yielded higher compared to prior to fermentation. Betacyanins are able to bind several molecules of sugars (glucose,

fructose, galactose, arabinose and several other molecules of sugar, such as disaccharides and polysaccharides, and flavonoid compound present in polyphenol group (Welch *et al*, 2008) which have possibility activity of anti bacteria. Declining total solids, polyphenol, betacyanin, dissolved protein, and pH are occurred by increasing total acids causing its occurrence of dilution of the whole components by chemical reaction on biomass.

Table 1: Materials composition as a result of purification of biomass of fermented beetroot through MF membrane for natural anti-bacteria.

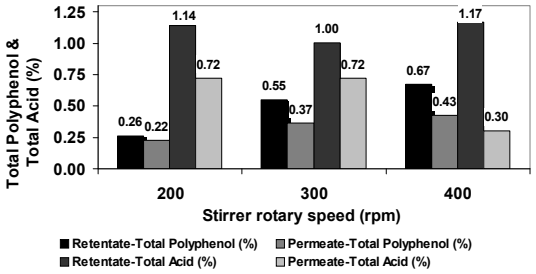
Components	Kind of materials		
	Pulp of beetroot*	Fermented beetroot 0 day*	Fermented beetroot 12 day*
Betacyanin (µg/mL)	0.43	0.45	0.060
Polyphenol	0.45	0.43	0.30
Total acids (%)	0.06	0.16	0. 50
Reducing sugars (mg/mL)	9.45	14.66	17.33
Total solids (%)	10.07	10.22	10.02
Dissolved protein (mg/mL)	21.41	21.20	15.36
pH	6.38	4.32	3.64

Legend: *ratio of beetroot/water (1: 8, w/w), **fermentation for 0 day, ***fermentation for 12 days.

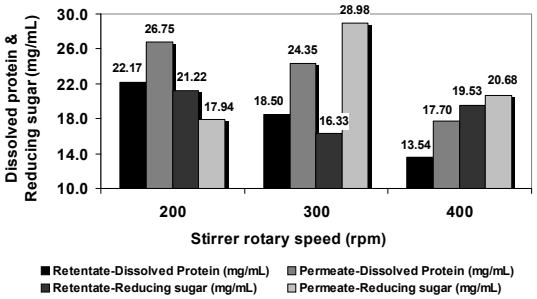
3.2 Influence on Process Condition of MF Membrane on Composition

3.2.1 Total Polyphenols (%), Total Acids (%), Dissolved Protein (mg/mL) and Reducing Sugars (mg/mL)

Separation process of biomass of beetroot at SRS 200, 300 and 400 rpm, and TMP 40 psia for 30 minutes generates retentate (concentrate) and permeate (extract) as clear liquid by degrading color from magenta to clear red. Increasing SRS is able to retain total polyphenols and total acids more much on the membrane surface compared to them passing in permeate, as shown in Fig 2a.



(a)



(b)

Figure 2: Effect of SRS at TMP 40 psia on (a) total polyphenols and total acids, and (b) dissolved protein and reducing sugars contents in retentate and permeate as a result of MF of fermented beetroot suspensions.

SRS becoming more and higher will increase total polyphenols in concentrate. This reason is possibily caused by its formation of 'cake' due to decreasing water mass passing through membrane so that it inhibits total polyphenol to pass through membrane (Mulder, 2012). Particle size of polyphenol compounds (MW between 200 and 600 Da.) (Liu, 2006), has smaller particle size than that pores size of MF membrane (0.15 µm), however, there is an interaction between SRS (200, 300 and 400 rpm) and fixed TMP 40 psia so that fouling phenomenon has occurred. Fouling is defined as the process in which microparticles, colloidal particles, solute molecules or bacteria trapped or accumulated on the membrane surface or into the membrane pores such that the membrane pores are blocked or become smaller, and formation of cake layer, in turn depletion of water mass so that particles of polyphenol and organic acids will be inhibited to pass across membrane. Polyphenol is chemical component having activity of anti-bacteria because of ability to increase membrane permeability from microbial cell so that membrane becomes unstabil causing cell hemolysis (Cushnie et al, 2005) Similar pattern is occurred at total acids, in which fouling is occurred in spite of particle size of organic acids compound (MW 200 – 250 Da.) are small (Liu, 2006). Organic acids is possibility have ability due to hydrophilic property from membrane so

that it functionates as transport media of positive charge ions. In other word, H+ diffuse to microbial cell wall (Trivedi *et al,* 2010) so that microbial cell wall will be more polar so that polyphenol compounds, flavonoid, etc. ease to permeate. On treatment combination at SRS 400 rpm and TMP 40 psia, optimization of total polyphenols and total organic acids in concentrate were achieved at 0.67% and 1.17% higher compared to passing in permeate 0.43% and 0.3%. In this condition, MF membrane system is able to retain total polyphenols and total acids in retentate 123.33% (1.23-folds) and 134% (1.34-folds), however, it passes both them 43.3% and 40% compared to total polyphenols (0.30%) and total acids (0.50%) in feed. SRS becoming higher, passes dissolved protein and reducing sugars to get optimization of MF membrane system followed by decreasing its concentration. This matter showed that MF membrane gives ability of separation unsuccessfully for both types of components, as indicated in Fig. 2b. Passing level of dissolved protein and reducing sugars are related with particle size calculated as amino acids and monosaccharides (0.01 – 0.1 µm) (PCI Membrane, 2005); Michael 1989) being smaller compared to MF membrane (0.15 µm), TMP (40 psia), and type of biomass. Interactions amongs these factors, it is not possibility occurred fouling so that they pass more much in permeate compared with being retained in retentate. Dissolved protein is the whole proteins from raw material of beetroot tuber (1.61%), in which beetroot tuber is degraded by microbe activity in Kombucha culture to dissolved protein derivatives accoding to Lowry (Lowry, 1951), whereas, reducing sugar is molecule of sugar having property of reducing because reactive hydroxyl ion (OH) according to Nelson-Somogyi method (AOAC 2019). Reducing sugars and dissolved protein become parameter in converting carbohydrate and protein by activity of microbes in Kombucha culture (Wong, 2015). Optimization of dissolved protein (22.17 mg/mL) and reducing sugars (21.22 mL) in concentrate is obtained at SRS 200 rpm, and passes dissolved protein (26.75 mg/mL) and reducing sugars (17.94 mg/mL) in permeate. In this optimum condition, MF membrane system is able to retain dissolved protein (44.33%) and reducing sugars (22.45%) in retentate, however, it passes dissolved protein 74.15%) and reducing sugars (3.40%) in permeate compared to dissolved protein (15.36 mg/mL) and reducing sugar (17.33 mg/mL) in feed.

3.2.2 Betacyanin and Total Solid

Betacyanin in biomass of beetroot, retentate and permeate as a result of MF process are possibility as compound being have activity of anti bacteria in phenolic compound group (Coultate, 2009). Separation process of betacyanin and total solids from biomass of beetroot showed that increasing SRS generates increasing betacyanin until it is get the best SRS followed by dropping betacyanin, however, on total solids becomes more and more high to optimum SRS. The best SRS on betacyanin was achieved at 300 rpm, which is able to separate betacyanin in retentate 0.31 µg/mL and passes in permeate 0.16 µg/mL, as shown in Fig. 3a. In the optimum condition, MF membrane separates betacyanin in retentate 416.67% (4.17-folds) compared to betacyanin in feed (0.06 µg/mL). It had been known that fermentation process declines betacyanin by effect of glucosidase enzyme of Kombucha culture (Havlíková et al, 1983). With particle size ranging from 0.001 – 0.01 µm, like flavonid compounds passes freely in permeate, however, due to presence of fouling causes betacyanin trapped in 'cake' layer on the membrane surface. On total solids, MF membrane showed increasing total solids in line with incresing SRS, as showed in Fig. 3b.

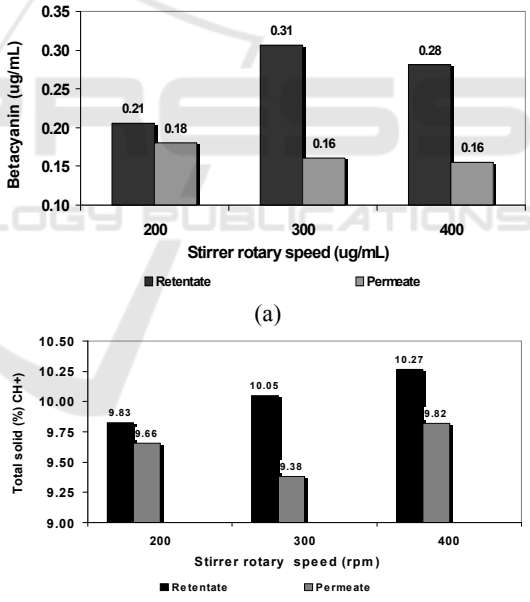


Figure 3: Effect of SRS at TMP 40 psia on (a) betactanin and (b) total solids contents in retentate and permeate as a result of MF of fermented beetroot suspensions.

MF membrane process generates a separation successfully, in which total solids retained on the membrane surface in retentate much more than that passing in permeate for the whole process treatment. Increasing total solids is caused by deficit of water mass passing across membrane as a consequence

from interaction between driven force and SRS to solidify components on the membrane surface. Optimization of total solids is achieved by retentate (10.27%) higher compared to pass in permeate (9.82%) at SRS 400 rpm. In this optimum condition, MF membrane process is able to retain total solids in retentate 2.49%, however, pass in permeate 1.99% compared to total solids in feed (10.02%). Total solids is an accumulation of the whole components of beetroot biomass both soluble and insoluble in water according to Gavimetric method (AOAC 2019) [17].

3.3 Optimum Condition Process of Microfiltration

Based on the highest betacyanin concentration as prevention of the best natural infection, separation process of betacyanin from fermented beetroot was achieved at SRS 300 rpm and TMP 40 psia. In this condition is yielded concentrate and permeate of fermented beetroot with composition of betacyanin 0.31 and 0.16 µg/mL, total polyphenols 0.55 and 0.37%, total acids 1.00 and 0.72%, reducing sugars 16.33 and 28.97 mg/mL, total solids 10.05 and 9.38%, and dissolved protein 18.50 and 24.35 mg/mL. In this condition, MF membrane process is able to retain components in concentrate and pass components in permeate on betacyanin 416% (4.16-folds) and 166% (1.67-folds), total polyphenols 83.33% and 23.33%, total acids 100% (1-fold) and 44%, reducing sugars 6.31% and 88.61%, total solids 0.3% and 6.84%, and dissolved protein 20.44 and 58.53% compared to concentration of components in feed. Fig. 4 shows feed (biomass of beetroot fermented for 12 days), concentrate and permeate yielded from MF membrane process at room temperature, SRS 300 rpm and TMP 40 psia for 30 minutes.

(a) (b) (c)

Figure 4: (a) feed, (b) retentate and (c) permeate as a result of MF of fermented beetroot suspension at optimum condition (300 rpm and 40 psia).

3.4 Activity of Anti Bacteria from Fermented Beetroot

Fermented beetroot has possibility activity of natural anti bacteria relating with presence of polyphenol compounds, particularly betacyanin. Retentate from MF membrane process on fermented beetroot at optimum condition (SRS 300 rpm, TMP 40 psia) indicates an ability in inhibiting the growth of *Staphylococcos aureus* and *Escherichia coli* on Agar Sodium with concentration 10 µmL incubated at 37 °C for 18 hours. Fig. 5a and 5b displays ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* in retentate of fermented beetroot with diameter of the inhibition zone of 11 mm and 10 mm, respectively.

(a) (b)

Legend: *Number 20 and 21 are code of samples.

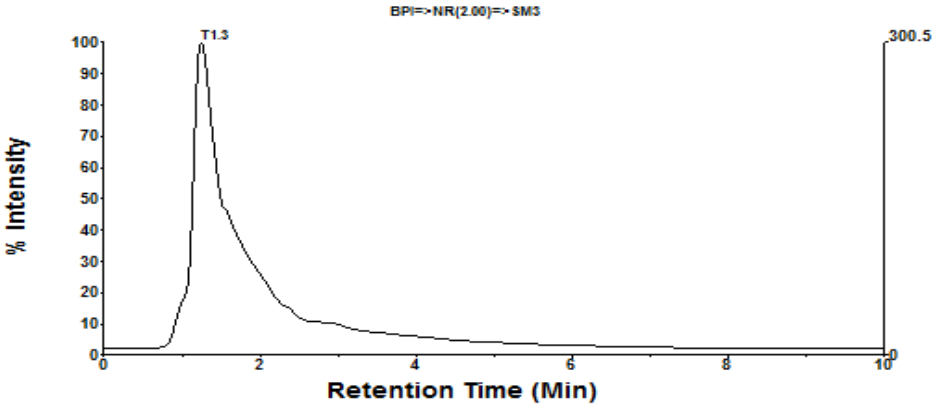
Figure 5: The growth of inhibition ability of (a) *Staphylococcus aureus* and (b) *Escherichia coli* from retentate of fermented beetroot suspension at optimum condition.

Feed and permeate does not demonstrate presence of clear zone arounds colony of *Staphylococcus aureus* and *Escherichia coli* or in other words it does not display activity of anti bacteria. This matter showed that MF membrane process tends to affect on ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* according to concentrations of total polyphenol (0.55%) and betacyanin (0.31 µg/mL) at higher concentrate compared to biomass without through MF membrane process with total polyphenol (0.30 µg/mL) and betacyanin (0.06 µg/mL). Similar case is also occurred in permeate according to both lower concentration of total polyphenol (0.37%) and betacyanin (0.16 µg/mL) compared to retentate. Bioactive compound concentration becoming more and more high will increase its activity as anti- bacteria (Brooks et al 2010). In general, S. aureus as a gram-positive bacterium has cell wall composed from peptidoglycan, in which polyphenol has property of toxis so that it is able to inhibit bacterial adhesin, Enzymes function and protein transport on cell cover (Cowan, 1999). that has deleterious ability on bacteria function causing lysis.

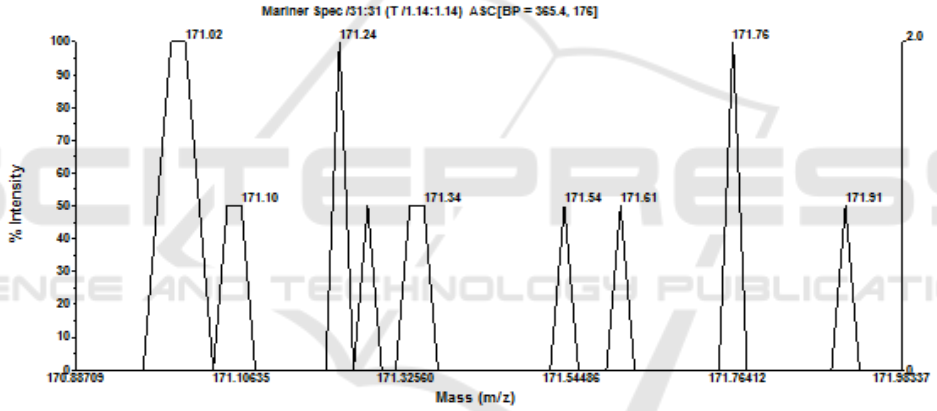
3.5 Identification of Monomer on Polyphenol and Betacyanin Compounds

Identification on polyphenol and betacyanin monomers is performed on an aliquot of retentate and

permeate from optimum condition treatment at SRS 300 rpm and TMP 40 psia by means of LC-MS based on molecular weight (MW) of gallic acid (MW 170 Da.) and betacyanin (MW 550 Da.). By means of 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+ [12]. Operation condition of LC-MS was adjusted in column of C-8 (15 mm x 2 mm), in which mobile phase (eluent) is methanol solution at flow rate of 0.1 mL/minute and injection volume of 5 µL, as displayed in Fig 6a-6f.



(a)



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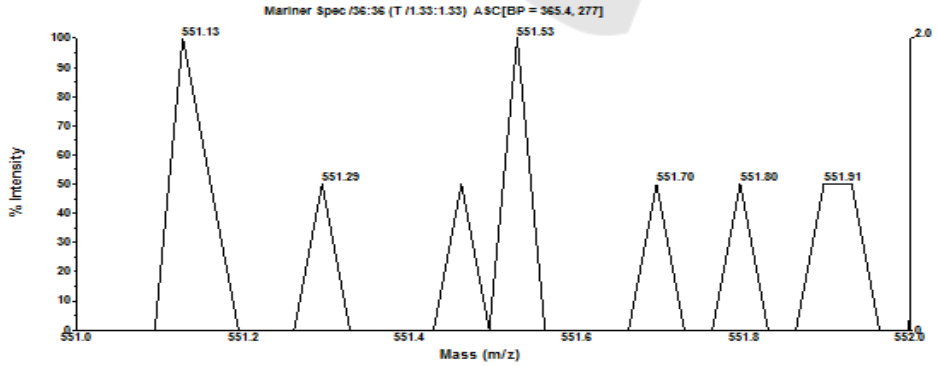


(c)

Figure 6: (a) Chromatogram of retentate, (b) mass spectra of retentate monomer as gallic acid, (c) mass spectra of retentate monomer as betacyanin, (d) chromatogram of permeate, (e) mass spectra of permeate monomer as gallic acid, (f) mass spectra of permeate monomer as betacyanin as a result of MF of fermented beetroot suspensions at room temperature, SRS 300 rpm, and TMP 40 psia for 30 minutes.

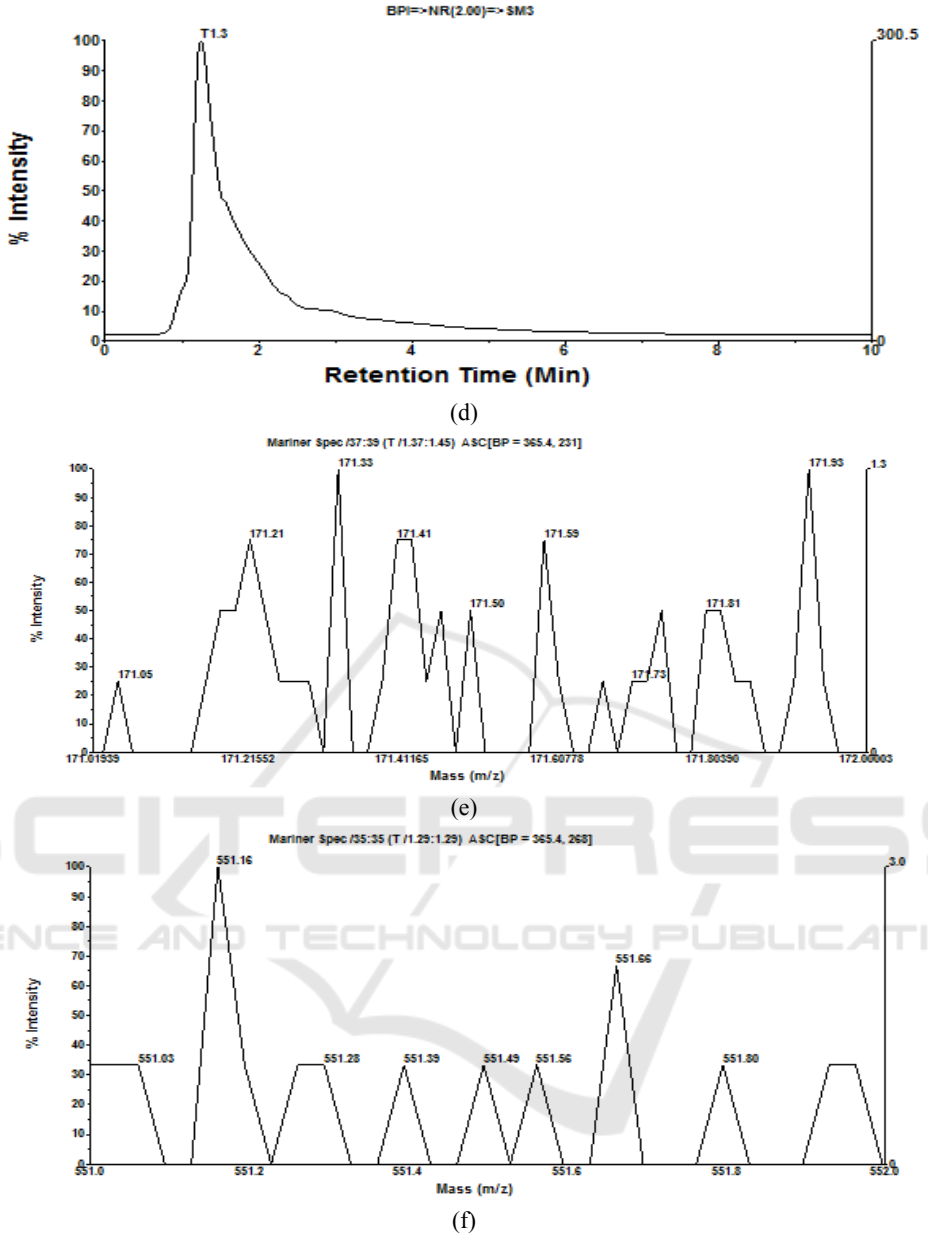


Figure 6: (a) Chromatogram of retentate, (b) mass spectra of retentate monomer as gallic acid, (c) mass spectra of retentate monomer as betacyanin, (d) chromatogram of permeate, (e) mass spectra of permeate monomer as gallic acid, (f) mass spectra of permeate monomer as betacyanin as a result of MF of fermented beetroot suspensions at room temperature, SRS 300 rpm, and TMP 40 psia for 30 minutes (cont.).

On polyphenol monomer as gallic acid, chromatogram on fermented beetroot concentrate shows one (1) peak (T1.3), in which at mass spectra T1.3 is get 8 monomer of gallic acid with MW between 171.02 and 171.92 Da. dominated by 3 monomers of gallic acid with MW 171.0231, 171.24, and 171.76 Da. (2M+) and relative intensity of 100%. Meanwhile, on monomer of betacyanin is get 6 monomers of betacyanin with MW between 551.13 and 551.91 Da. dominated by 2 monomers of betacyanin with MW 551.13 and 551.53 Da. and relative intensity 100%, as shown in Fig 6a, 6b, and 6c. Permeate of fermented beetroot is get chromatogram with one (1) peak with mass spectra 10 monomers of gallic acid dominated by monomer with MW 171.23 and 171.72 Da. with relative intensity 100%, whereas monomer of betacyanin is get 9 monomers of betacyanin with MW between

551.03 and 551.95 Da. dominated by monomer of betacyanin with MW 551.16 Da. and relative intensity 100%, as showed in Fig 6d, 6e and 6f.

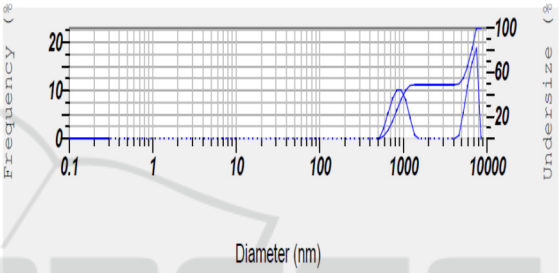
3.6 Distribution of Particle Size

Based on betacyanins, MF membrane process on fermented beetroot with the best process condition is resulted from concentrate with thick suspension and permeate with clear liquid by degrading colour from red to dark red. By using stirrer at SRS 300 rpm and TMP 40 psia for 30 minutes affects possibility on particle size and particle size distribution. Particle size and particle size distribution are conducted to know the characteristic of suspension relating with adsorption aspect in the digestive system. Table 2. demonstrates larger particle size of feed (5656.4 nm) than particle size of retentate (3002.4 nm) and permeate (1962.0 nm) with particle index of 0.370, 1.912 and 0.468, respectively. The difference in this particle size is possibily caused by MF system being separating suspension so that biomass feed without MF system has the highest particle size. Meanwhile, the concentrate is an accumulation from all components with particle size larger than 0.15µm due to fouling phenomenon and permeate has the smallest particle size because components with particle size smaller than 0.15µm passes freely in permeate. Dispersion of particles are displayed as dispersed particle index (PI), in which feed has the highest PI (1.912) compared to PI of retentate (0.468) and PI of permeate (0.370). Particle size becoming more and more small will be small in PI or in other words dispersion of particle is more uniform and homogen. It had been appeared that retentate and permeate have PI smaller than 1 expressed that particle size distribution is more homogenous compared to feed indicating particle size distribution is ununiform (PI > 1) (Eichhorn, 2001).

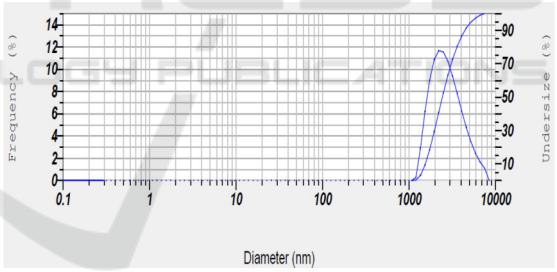
Table 2: Characteristic of particles in feed, retentate and permeate by MF membrane fitted in DESMFC at room temperature, SRS of 300 rpm and TMP 40 psia for 30 minutes.

Kind of fermented beetroot*	Distribution of nano-polyphenol particles (nm)	
	Z-Average (nm)**	PI***
Feed*	5656.4	1.912
Retentate (concentrate)	3002.4	0.468
Extract (permeate)	1962.0	0.370

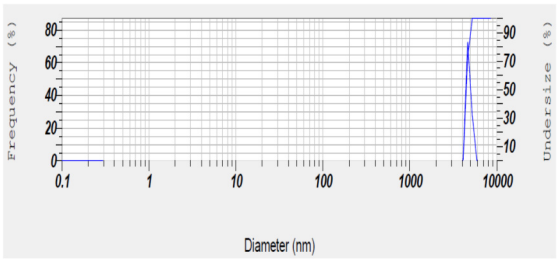
Particle size distribution on feed showed that particles have diameter size (Ø) of 500 – 1200 nm and 700 – 9000 nm (> 10000) at frequency between 0 and 10%, and between 0 and 15% or the whole particles with Ø between 500 and 10000 nm (> 10000 nm) at frequency between 0 and 25%, respectively, as indicated in Fig 7a. It had been appeared that particles in retentate have Ø between 1000 and 9500 nm (> 10000 nm) at frequency between 0 and 13% or the whole particles with Ø between 1000 and 9500 nm (> 10000 nm) at frequency between 0 and 15%, as demonstrated in Fig. 7b. Meanwhile, on permeate appears particles with Ø between 6000 and 8000 nm at frequency between 0 and 65%, as shown in Fig. 7c. Particle size distribution indicates a difference in the whole materials, in which feed has particle size with more variation compared to retentate and permeate.



(a)



(b)



(c)

Figure 7: Particle size distribution of (a) feed, (b) retentate, and (c) permeate as a result of separation of fermented beetroot by MF membrane fitted DESUFC at room temperature, SRS of 300 rpm and TMP 40 psia for 30 minutes.

4 CONCLUSIONS

Separation of total polyphenol and betacyanin compounds from fermented beetroot through MF membrane generates a separation successfully for betacyanin, total solids, total acids, and unsuccessfully for dissolved protein and reducing sugars. SRS becoming more and higher will retain total polyphenol, betacyanin, total acids, total solids in retentate, however SRS pass freely dissolved protein and reducing sugars in permeate. Based on recovery of betacyanin, optimization on process condition by means of MF technique was achieved at SRS 300 rpm being resulting retentate and permeate with composition betacyanin of 0.31 and 0.16 µg/mL, total polyphenols of 0.55 and 0.37%, total acids of 1.00 and 0.72%, reducing sugars of 16.33 and 28.97 mg/mL, total solids of 10.05 and 9.38%, dissolved protein of 18.50 and 24.35 mg/mL, particle size 3002.4 and 1962.0 nm, particle index 0.468 and 0.370, respectively. Identification on betacyanin and galic acid monomers as total polyphenol at retentate is dominated by monomer with molecular weight (MW) 551.13, 551.53, and 171.02, 171.24 and 171.76 Dalton (Da.), meanwhile permeate is dominated by monomer with MW 551.16 and 171.23, 171.72 Da. and relative intensities 100%, respectively. Ability to inhibit the growth of bacteria of *Staphylococcus aureus* Ina CC-B4 and *Escherichia coli* Ina CC-B5 is obtained by retentate with zone area of inhibiting 11 and 10 mm, respectively. In this optimum condition, MF membrane technique was able to retain betacyanins 416% (4.16-folds), total polyphenol 83.33%, total acids 100% (1-fold), reducing sugars 6.31%, total solids 0.3% and dissolved protein 20.44% in retentate, whereas it passes betacyanin 166% (1.67-folds), total polyphenol 23.33%, total acids 44%, reducing sugars 88.61%, total solids 6.84% and dissolved protein 58.53% in permeate compared to components prior to process (feed).

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