Recovery of Fermented Spinach (*Amaranthus* sp.) Concentrate Through Ultrafiltration Membrane as Source of Folic Acid

Aspiyanto*, Agustine Susilowati, Puspa Dewi Lotulung, Hakiki Melanie, and Yati Maryati

Research Center for Chemistry – Indonesian Institute of Sciences, Kawasan PUSPIPTEK, Serpong – 15314, South Tangerang, Banten, Indonesia

Received May 22, 2017; Accepted August 23, 2017

ABSTRACT

*Fermentation process on spinach (*Amaranthus sp.*) by Kombucha culture was done as an effort to recover naturally folic acid as bioactive components to increase smartness. The experimental activity was done by means of UF membrane (100,000 MWCO) fitted in Stirred Ultrafiltration Cell (SUFC) at stirrer rotation speed 200 and 400 rpm, room temperature, pressure 20 and 40 Psi for 30 min. Result of experimental activity showed that based on both selectivity and recovery of folic acid, process optimization of UF was reached at stirrer rotation speed 200 rpm and pressure 40 Psi. In the optimum condition, SUFC technique was able to recover folic acid in retentate 67.75% and in permeate 97.27% (63.19 µg/mL). Identification of monomer in permeate from the optimum process treatment was find out folic acid monomer with molecular weight (MW) 441.39 and relative intensity 93% at mass spectra T2.32 between m/z 257–304 and glutamic acids monomer with MW 148.57 and relative intensity 0.22% at mass spectra T2.82 between m/z 415–470. Other dominant monomer were folic acid fraction.*

Keywords: *fermented spinach (*Amaranthus sp.*); Stirred Ultrafiltration Cell (SUFC); permeate; concentrate; folic acid*

ABSTRAK

*Proses fermentasi pada bayam (*Amaranthus sp.*) oleh kultur Kombucha dilakukan sebagai upaya untuk memperoleh asam folat alami sebagai komponen bioaktif untuk kecerdasan. Penelitian ini dilakukan dengan menggunakan membran Ultrafiltrasi (UF) yang dipasang pada Sel Ultrafiltrasi Berpengaduk (SUFB) pada kecepatan putar pengaduk 200 dan 400 rpm, suhu ruang serta tekanan 20 dan 40 Psi selama 30 menit. Hasil penelitian menunjukkan bahwa optimisasi proses UF berdasarkan selektifitas dan perolehan kembali asam folat dicapai pada kecepatan putar pengaduk 200 rpm dan tekanan 40 Psi. Pada kondisi optimal ini, SUFB mampu memperoleh kembali asam folat 67,75% dari asam folat dalam retentat dan dalam permeat (63,19 µg/mL) dengan tingkat rejeksi 97,27%. Identifikasi monomer pada permeat dari perlakuan proses optimal ditemukan monomer asam folat berberat molekul (BM) 441,39 dengan intensitas relatif 93% pada spektra massa T2.32 antara m/z 257–304 dan monomer asam glutamat berberat molekul (BM) 148,57 dengan intensitas relatif 0,22% pada spektra massa T2.82 antara m/z 415–470. Monomer dominan lainnya berupa pecahan asam folat.*

Kata Kunci: *bayam (*Amaranthus sp.*) terfermentasi; Sel Ultrafiltrasi Berpengaduk (SUFB); permeat; konsentrat; asam folat*

INTRODUCTION

Fermented spinach (*Amaranthus* sp.) by Kombucha culture are the novelty fermented process as an effort to recover folic acid, folate, folacin, vitamin B9, and pteroyl-L-glutamic acid for functional food based on nutrition need of smartness enhancer and anemia prevention [1-2]. Exploring folic acid on spinach will place such commodity into alternative recovery of folic acid. Fermentation on spinach by Kombucha culture will be occurred like on Kombucha tea (*Camellia sinensis*), which is able to increase polyphenol, organic acids (acetic, lactic, etc.), vitamin (vitamin B12, vitamin B9 as folic acid) and amino acids [3] with specific aroma yielded through assimilative and dissimilative chemical reactions during 7– 4 days [4].

Recovery of folic acid through fermentation of spinach by Kombucha is occurred due to activity of Kombucha culture through biosynthesis [5] on amino acids (adenine, histidine, methionine) both on Kombucha culture (fungi, bacteria, yeast) and leaves. This biosynthesis provides enzyme activities of dihydrofolate synthase (DHFS) and folypolyglutamate synthase (FPGS) to form dihydrofolate and polyglutamate folate, respectively, which are form of

* Corresponding author. Tel : +62-21-7560929
Email address : aspiyanto_2010@yahoo.com

DOI: 10.22146/ijc.25165

folic acid derivatives [5], in which this process occurred on plants and microbes [6].

Biomass of fermented spinach product able to be concentrated through ultrafiltration (UF) membrane (100,000 MWCO) fitted in Stirred Ultrafiltration Cell (SUFC) to recover concentrate as source of folic acid fortified into food product. Folic acid is known for instability on light, oxygen, mechanic treatment and high temperature. Therefore, alternative method is required. One of the potential alternative methods to minimize loss of folic acid is concentration process biomass of fermented spinach by means of UF membrane. Stirred Ultrafiltration Cell (SUFC) operates based on difference in molecular weight (MW) [7], in which larger MW components than pore size of membrane will be retained as retentate. On the other hand, smaller MW components or smaller particle size than pore size of membrane will freely pass through membrane as permeate [8-9].

By using UF membrane (100,000 MWCO) fitted in SUFC mode, folic acid MW (441 Da.) will possibly pass through as permeate, however, due to its presence of 'fouling', folic acid particles will be trapped on retentate side as 'cake' layer. Using dead-end SUFC mode, fluid flows across and through a membrane (as filter media) and solute particles are retained on the top membrane surface, as a consequence, feed fluid flows through membrane and cake layer on the top membrane surface [10].

Performance of UF membrane in terms of flux and rejection coefficient is strongly effected by operation condition (stirrer rotation speed, temperature, pressure, time), and correlated to the both material type and concentration. In UF process application, an ideal membrane performance criteria in concentrating process by means of UF is permeate flux value and rejection factor (R) [9]. Rejection factor (R) of a given component by a membrane is usually defined as one minus the ratio of the any component concentration permeate to the any component concentration retentate [11], expressed as $R = 1 - C_p/C_r$, where C_r is the concentration of component in the retentate and C_p is the concentration of component in the permeate [12].

The purpose of this experimental activity was to find out component compositions, particularly folic acid and monomer characteristic from retentates (concentrates) and permeates as a results of concentration process of spinach fermented by Kombucha culture as functional food for smart food through UF membrane (100,000 MWCO) fitted in SUFC at fixed process condition (stirrer rotation speed 200 and 400 rpm, room temperature and pressure of 20 and 40 Psi for 30 min).

EXPERIMENTAL SECTION

Materials

Main materials used in this experimental activity were distilled water, fresh spinach (*Amaranthus* sp.), sucrose purchased from local market, Kombucha culture (Research Centre for Chemistry – LIPI), ultrafiltration (UF) membrane 100,000 MWCO (Fluoropolymer, FSM-0.15-PP, Alfa Laval, Denmark), and chemical reagents for process and analyses purposes. While, all chemical reagents used were of analytical grade or purer, and solutions were based on highly purified water (distilled water).

Instrumentation

Main equipments utilized in this experimental activity were fermentation system, laminar flow system, water bath (Memmert, Germany), incubator, cylindrical tank for technical nitrogen, pressure gauge of technical nitrogen (Fisher Scientific Company, England), Dead-End Stirred Ultrafiltration Cell (SUFC) (MILLIPORE, Model 8200, USA), UV-Vis Spectrophotometer (Model RF-550, Shimadzu, Japan) and LC-MS (Mariner Biospectrometry) with LC (Hitachi L 6200).

Experimental Design and Analysis

Experimental work was conducted by using biomass filtrates of fermented spinach by Kombucha culture sieved through 100 mesh was further purified by means of MF membrane (0.15 µm) equipped in SUFC [13] to produce retentate (concentrate) and permeate. Analysis were performed on total solids (Gravimetric method), dissolved protein (Lowry method) [14], N-amino (Copper method) [15], total polyphenol (Folin-Denis Method) [16], folic acid (spectrophotometry UV-Vis) [17]. Identification on monomer biomass was conducted by LC-MS with LC [18]. Process and analysis were conducted in duplicate. Data were processed in this description based on result of average analysis.

Procedure

Preparation of inoculum and fermentation process of spinach

A number of fresh and clean spinach was blanched at 80 °C for 15 min, pulverized at a 1:4 ratio of blanched spinach to water (sterile) until spinach suspension is reached. Blanched spinach was sieved through 100 mesh to yield filtrate (Fig. 1a) and residue.

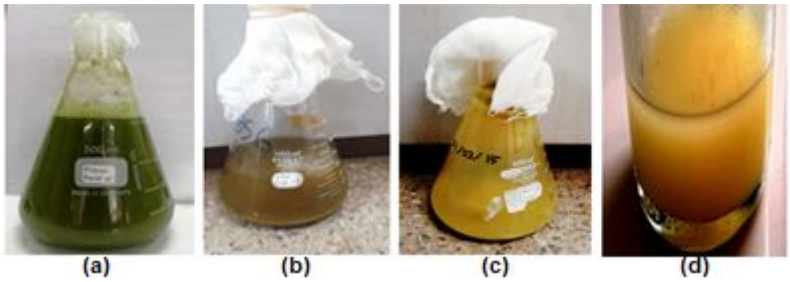

Fig 1. (a) Spinach filtrate through 100 mesh sieve as a result of pulverizing at a 4:1 ratio of blanched spinach (80 °C, 15 min) to water (sterile), (b) biomass of fermented spinach (1 day), (c) biomass of fermented spinach (3 days) and (d) fermented spinach concentrate produced by filtered of MF (0.15 µm)

A number of spinach filtrate was further added sucrose (10%, w/v vegetable filtrate) and Kombucha culture (15% (v/v), and fermented in closed container using casa cloth in dark room at room temperature for 5 days. Product of fermentation process is biomass of fermented spinach (Fig. 1b and 1c). All fermentation activity was aseptically. Recovery of each biomass is introduced as feed in purifying process of folic acid through UF membrane (100,000 MWCO).

Separation of fermented spinach by Stirred Ultrafiltration Cell (SUFC)

A number of biomass of fermented spinach suspension was filtered through 100 mesh sieve, and ultrafiltered via UF membrane (100,000 MWCO) equipped in SUFC at stirrer rotation speed 200 rpm, room temperature and pressure 40 Psi for 30 min. Both same method and process condition are done for stirrer rotation speed 400 rpm, room temperature and pressure 40 Psi for 30 min. The commercial UF membrane disc having a 100,000 MWCO with the glossy skin side toward solution was placed into the Stirred Ultrafiltration Cell (SUFC) and connected to a nitrogen gas cylinder. A SUFC of 180 mL capacity and a membrane area of 30.175 cm^2 was operated in the filtration experiments [9]. Each MF membrane was first compacted at 40 Psi for 5 min. After that, operation of SUFC was stopped and distilled water was drained. The suspension of fermented spinach filtrate was added to the SUFC. Operation conditions were stirrer rotation speed of 400 rpm and trans-membrane pressure (TMP) of 40 Psi for 30 min. All MF experiments were done at a room temperature. Volumetric permeate flow rate was measured after each mL of permeate collected. Samples of permeate were collected and recorded for 30 min [13]. Permeate and retentate (concentrate) produced by microfiltered of membrane of 0.15 µm (Fig. 1d) were regularly sampled for analysis of their folic acid, total solids, dissolved protein, amino acids, reducing sugar and kind of monomer by LC-MS.

Identification of folic acid, glutamic acid and polyphenol in fermented spinach via LC-MS

Sample of fermented spinach extract was microfiltered using 0.15 µm MF membrane equipped in SUFC at room temperature for 30 min until it yields permeate and retentate (concentrate) [11]. Permeate was collected and introduced as material sample for identification of protein isolate monomer through LC-MS using Mariner Biospectrometry. LC system was integrated with Q-tof mass spectrometer through Electro Spray Ionization (ESI) system, in which scan mode was done at range of m/z 100–1200 and 140 °C. LC (Hitachi L 6200) uses a C18-18 RP (5 µm particle size, 25 cm x 1 mm i.d.) column from Supelco (Bellefonte, PA). Kinds of solvent were water containing 0.3% acetic acid (A) and methanol containing 0.3% acetic acid (B) at a 90 parts of methanol : 10 parts of water ratio at flow rate 1 mL/min and injection volume of 20 µL [18].

RESULT AND DISCUSSION

Characteristic of Material

Feed introduced in this experimental work was fermented spinach retentate produced through 0.15 µm MF membrane at stirrer rotation speed 400 rpm, room temperature and pressure 40 Psi for 30 min. Fermented spinaches were produced through a series of process including blanching (80 °C, 15 min), pulverizing at ratio of 1 part blanched spinach to 4 part of water (sterile), filtered through a 100 mesh sieve and fermented by Kombucha culture in closed container in dark room at room temperature for 3 days [19]. Fermentation becoming more and longer increase composition of component, and purification process of biomass through a 0.15 µm MF membrane fitted in SUFC mode was obtained a concentrate as thick suspension with brownish yellow color, as displayed in the whole compositions in Table 1. This process produce recovery of folic acid in concentrate (93.09%) compared to initial folic acid [19].

Table 1. Composition of vegetable materials as feed and result of purification of folic acid in fermented vegetables through 0.15 µm MF membrane

Kind of material	Compositions				
	Total solid (%)	Total Polyphenol (%w/w)	Folic acid (µg/mL)	Reducing sugar (mg/mL)	Total acid (%)
Blanched spinach*	5.04	0.69	14.90	17.04	0.10
Fermented spinach (1 day)	9.50	0.04	22.86	46.68	0.19
Fermented spinach (3 days	7.90	0.07	54.83	86.30	0.58
Fermented spinach concentrate**	6.29	0.25	58.90	105.34	0.57

Legend: *at 80 °C &15 min, **result of microfiltration of 0.15 µm as feed

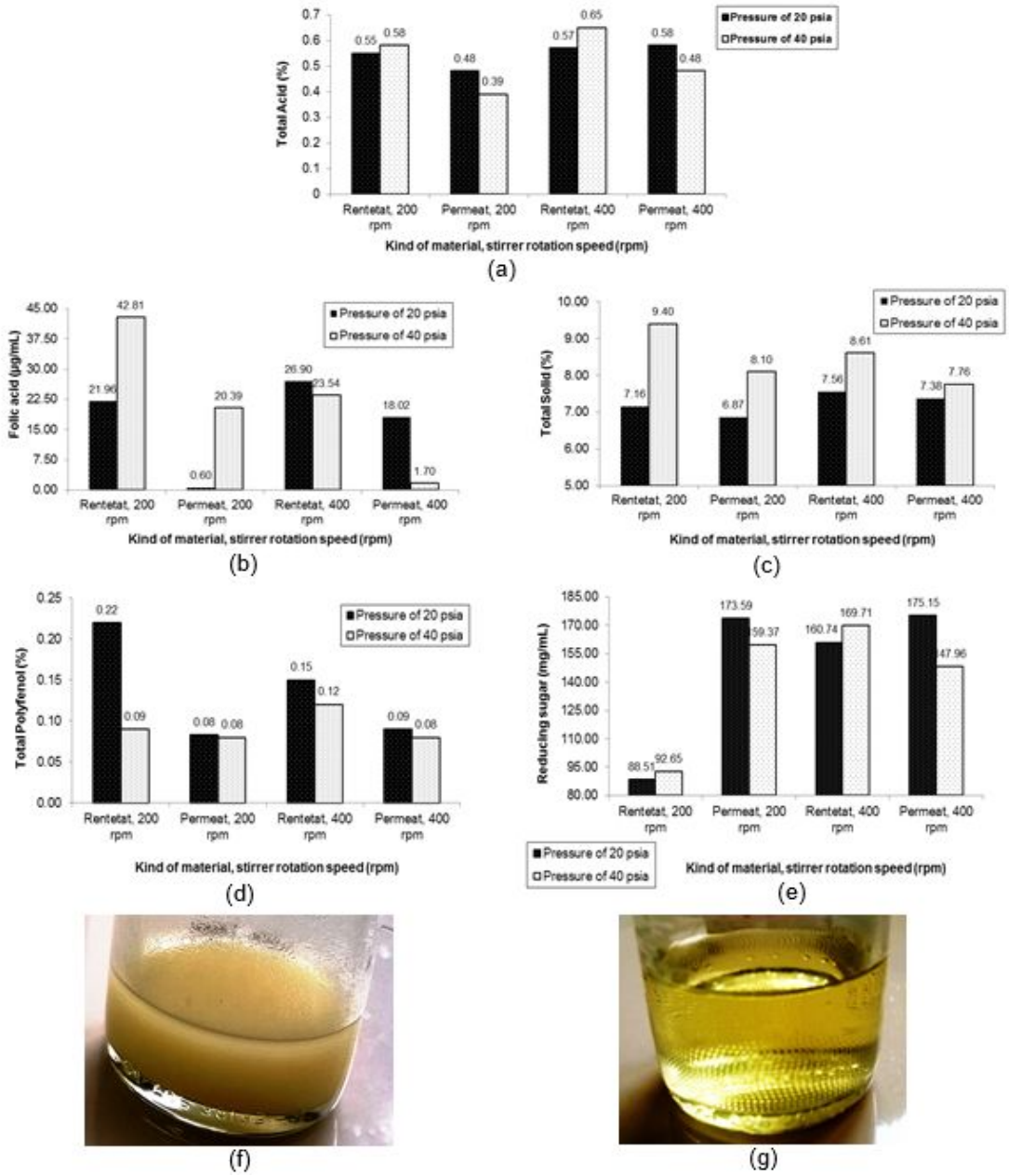

Fig 2. Relationship between stirrer rotation speed on contents of (a) total acids, (b) folic acid, (c) total solids, (d) reducing sugar and (e) total polyphenol in retentate and permeate of fermented spinach biomass yielded by UF membrane (100,000 Da.) at room temperature for 30 min, (f) concentrate and (g) permeate of fermented spinach produced by concentration process at stirrer rotation speed 200 rpm and pressure 40 Psi

Effect of Concentration Process of Fermented Spinach by Ultrafiltration (UF) on Composition

Lactic, malic, acetic acids, etc. counted as total titertable acids are parameter in which fermentation of spinach by Kombucha culture had been occurred. These acids are metabolite produced by activity of Kombucha culture to ferment sucrose (10%) as main source of carbon on microbes metabolism. Ultrafiltration (UF) operation under pressure 20 and 40 Psi with stirrer rotation speed 200 and 400 rpm was able to retain more much total acids in retentate than that passing in permeate for the whole process treatments, as shown in Fig. 2a. With low molecular weight (MW) (smaller than 150 Da.) and particles size of 0.001–0.01 μm causes high driven force needed to retain more much particles on the top membrane surface. These organic acids enables to pass freely more in permeate on using UF membrane of 100,000 MWCO. However, there are fouling during UF process causing more much organic acids was retained on the top membrane surface. Under both higher stirrer rotation speed and pressure (400 rpm, 40 Psi) causes stronger polarization of particles by higher driven force compared to the other process treatments. The best recovery of organic acids were achieved at stirrer rotation speed 400 rpm and pressure 40 Psi by retaining them in retentate 0.65% and passed them in permeate 0.48% with rejection factor 2.5%, as summarized in Table 2. This rejection factor is low (lower than 90%). In other words, SUFC mode is not optimum to concentrate this organic acids. In this condition, recovery of organic acids in retentate was 57% from total organic acids in retentate and permeates (1.13%).

Different trend seems on folic acid with combination between lower stirrer rotation speed and higher pressure (200 rpm, 40 Psi), so that the optimum SUFC in retaining folic acid (42.81 μg/mL) and passes it in permeate 20.38 μg/mL, as showed in Fig. 2b. In this condition, recovery of folic acid in retentate was 67.75% from folic acid in retentate and permeate (63.19 μg/mL) with rejection factor 97.27% (Table 2). High rejection factor was caused by high selectivity as a consequence of interaction between process treatment and internal factor of folic acid. Particles size of folic acid of approximately from 0.008 to 0.01 μm [20-21] with MW 441 Da. should more much passing in permeate, however fouling phenomena is occurred due to trapping and deposition of folic acid on 'cake' layer, a polarization of the whole particles on the membrane surface [19]. Fouling is occurred by specific interaction physically and chemically amongst various soluble solids on membrane [9,22] so that a part of particles pass in permeate.

This trend is similar on recovery of total solids, in which process treatment at stirrer rotation speed 200 rpm and pressure 40 Psi using SUFC mode is able to retain the optimum total solids in retentate (9.4%) and pass it in permeate 8.10%, as demonstrated in Fig. 2c. In this process, recovery of total solids in retentate is 53.71% from total solids in retentate and permeate (17.5%) with rejection factor 13.81% (Table 2). Low rejection factor is caused by high MW soluble and insoluble components, in which it had been known that biomass used is concentrate of MF (0.15 μm) product so that components with MW smaller 900 Da. are dominated by glutamic acids and its derivatives (MW 140–443 Da.), and microbes (yeast, fungi, bacteria) with particle size of 2–3 μm [19]. In other words, to concentrate total solids in biomass, SUFC mode operates unsuccessful. Total solid is the whole components (protein, carbohydrate, fat, and mineral) accumulated and material dissolved into water.

On total polyphenol, recovery optimization in retentate was reached by combining stirrer rotation speed 200 rpm and pressure 20 Psi, as displayed in Fig. 2d with rejection factor 61.31% (Table 2). This matter is caused by polyphenol biomass as feed produced from MF membrane has MW with range of 191–193 Da. and low relative intensity (1.5–1.91%) from the whole biomass monomer [19], so that it is occurrence of fouling will retain higher polyphenol (0.22%) in retentate compared to it pass in permeate (0.08%) causing the highest rejection factor compared to the other treatments. In this condition, recovery of total polyphenol in retentate is 66.67% from total polyphenol in retentate and permeate (0.3%). It had been known that polyphenol in vegetables have various MW (larger than 600 Da.) [16] or particles size with range of 0.001–0.01 μm [13]. Enzyme activity of catalase from fungi which is *Candida tropicalis* in Kombucha culture [3] degrade possibility polyphenol into catechin with lower MW during fermentation so that suspension has possibility antioxidant properties, like Kombucha tea [23].

Table 2. Rejection of components in biomass of fermented spinach on UF membrane

Pressure (Psi)	Stirrer rotation speed (rpm)	Rejection of components (%) on membrane 100,000 MWCO				
		Folic acid	Total acid	Total solid	Polyphenol	Reducing sugar
20	200	97.27	12.58	4.13	61.31	0
	400	33.01	0	2.41	43.37	0
40	200	52.39	33.11	13.80	8.16	0
	400	92.79	25.51	9.93	37.60	0

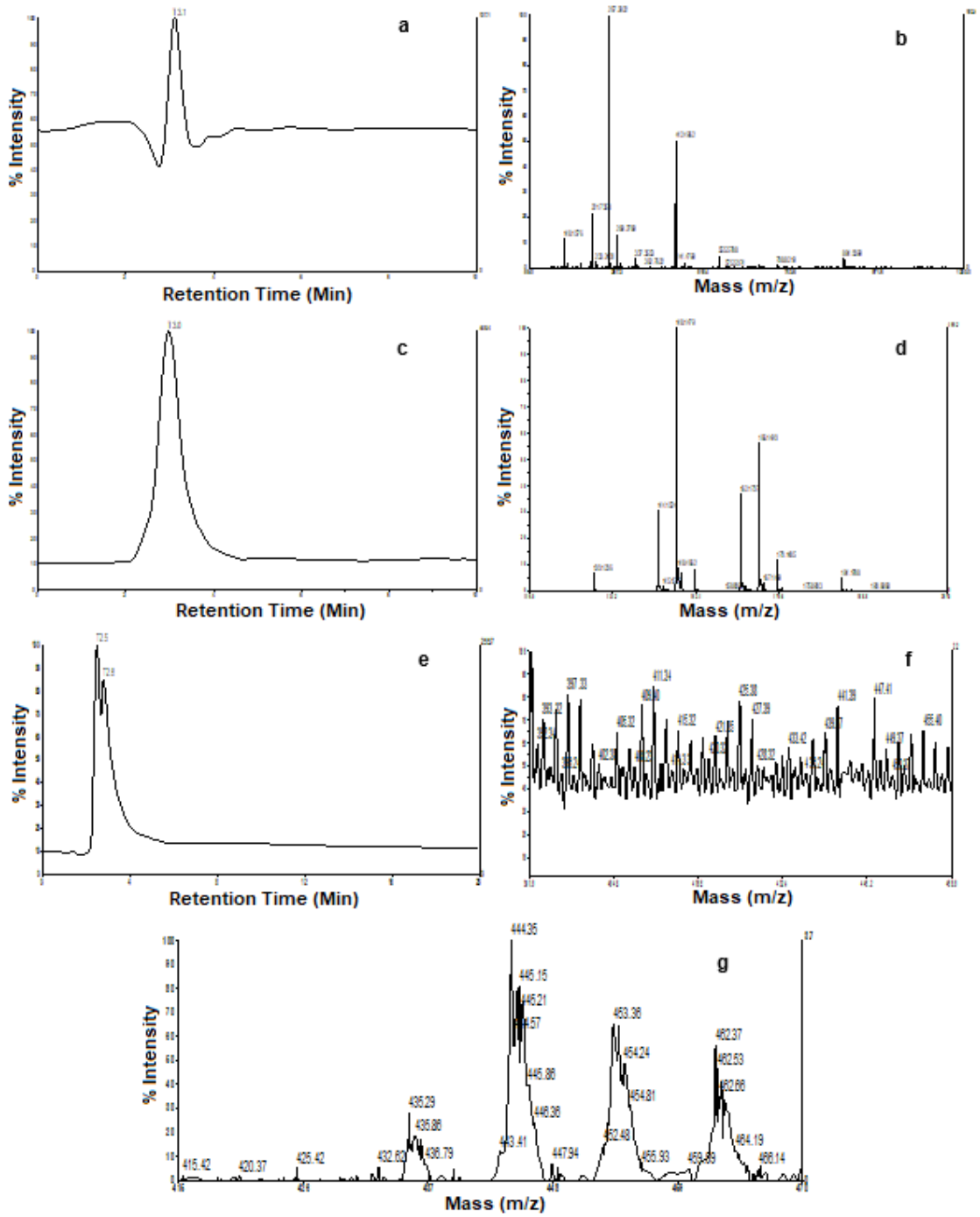

Fig 3. (a) Chromatogram of standard folic acid, (b) mass spectra T3.0 of standard folic acid, (c) chromatogram of standard glutamic acid, (d) mass spectra T3.0 of standard glutamic acid, (e) chromatogram of fermented spinach permeate, (f) mass spectra T2.32 of fermented spinach concentrate, (g) mass spectra T2.82 of fermented spinach permeate

Reducing sugar of biomass is non-fermented sugar remains by Kombucha culture after fermentation caused by internal factors of fermentation. The optimum SUFC mode operation in retaining reducing sugar (169.71 mg/mL) and passing in permeate of 147.96 mg/mL was shown in Fig. 2e. In this condition, recovery of reducing sugar in retentate 53.42% from folic acid in retentate and permeate (317.67 µg/mL) with rejection factor 0% (Table 2). In other words, SUFC mode operation is not able to concentrate reducing sugar successfully. Sugar

Table 3. Dominant monomer on mass spectra of standard folic acid

Index	Centroid Mass	Relative Intensity	Area	Index	Centroid Mass	Relative Intensity	Area
1	148.157631	11.37	95.19	21	289.278878	13.14	104.16
2	148.546075	1.34	15.39	24	337.355276	3.65	48.30
13	221.734988	21.69	177.84	26	442.496215	50.55	631.03
16	233.245128	2.27	13.33	27	443.166533	8.35	24.35
18	267.292185	100.00	1025.63	28	443.509427	14.98	60.46
19	268.291245	15.71	89.71	29	444.479853	2.44	78.59

Table 4. Dominant monomer on mass spectra of standard glutamic acid

Index	Centroid Mass	Relative Intensity	Area	Index	Centroid Mass	Relative Intensity	Area
2	144.152369	30.59	775.88	5	148.466240	8.51	56.76
4	148.147897	100.00	2542.26	6	149.145287	7.06	165.60

Table 5. Dominant monomer on mass spectra of extract of fermented spinach relating to standard folic acid and glutamic acid (T2.32 and T2.82)

Index	Centroid Mass	Relative Intensity	Area	Index	Centroid Mass	Relative Intensity	Area
29	148.112434	0.15	1.24	348	450.672045	0.07	3.95
30	148.291873	0.21	1.11	349	453.362772	2.13	7.13
31	148.570004	0.22	2.2	350	453.846091	2.12	2.99
338	443.411061	0.39	1.24	351	454.346611	1.58	2.06
339	444.352343	3.27	14.86	352	455.933404	0.17	1.46
340	444.867619	2.62	8.62	353	458.445519	0.11	2.17
341	445.149638	2.66	1.05	346	448.4262	0.17	1.50
342	445.359218	2.41	3.79	347	448.774166	0.10	3.04
343	445.864978	1.30	1.63	344	446.364662	0.78	1.05

particles have size in the range of 0.0008–0.001 µm (200–400 Da.) [21,24] so that they should pass more much in permeate except there is fouling. Another prediction is its occurrence of degrading to form simpler and smaller molecule units and pass as permeate. Reducing sugar is sugar molecule (monosaccharide or saccharide) having reducing properties because of reactive property of hydrosil (OH) [25] with high solubility in water.

From the whole descriptions, based on the highest rejection factor (97.27%) and the best folic acid concentration (42.81 µg/mL) in retentate (concentrate), the optimum treatment combination was achieved at stirrer rotation speed 200 rpm and pressure 40 Psi. Fig. 2f and 2g showed retentate (concentrate) and permeate as a yield of the optimum process condition at SUFC of fermented spinach.

Identification of Concentrate and Permeate Fermented Spinach Isolate Monomer

Chromatogram of standard folic acid and its mass spectra in Fig. 3a and 3b displayed dominant monomer with MW 267.2922 Da. and relative intensity 100% and there is a possibility as folic acid fractionation, while folic acid (MW 441 Da.) Monomer was showed with relative intensity 77%, as shown in Fig. 3a. Chromatogram of standard glutamic acid and its mass spectra were shown in Fig. 3c and 3d. Standard glutamic acids were subsequently dominated by compounds with MWs 148.15, 148.47 and 149.14 Da. and intensities 100, 8.51 and 7%, respectively. All dominant folic acid and glutamic acid monomers were tabulated in Tables 3 and 4. Folic acid and glutamic acid have MWs 441 and 148 Da. [26], however, since its presence of ionization caused by sensitivity of LC-MS instrument relating with eluent used causes its occurrence of difference in MW indicated by LC-MS. It had been known that a compound displays difference in MW, in which its presence a possibility as M^+, $M + Na^+$, $2\ M^{++}$ or $2\ M^+ + Na^+$ [18], so that standard folic acids were dominated by compounds with MW of 442.5, 443.16, 443.51 and 444.48 Da. and intensities of 50.55, 8.35, 15 and 2.44%. Operation condition of LC-MS is injection volume of 20 µL and flow rate 0.05 mL/min by using mixture methanol and water (containing 0.3% acetic acid) at ratio of 80:20 for both solution standards.

Based on the best recovery of folic acid, identification of fermented spinach concentrate monomer from process treatment at stirrer rotation speed 400 rpm and pressure 20 Psi yields chromatogram with 2 peaks (T2.32 and T2.8), as showed in Fig. 3e, 3f and 3g. Selection on permeate or extract in this analysis was performed due to glutamic acid and folic acid relating with low MW amino acids (200–500 Da.) or lower than 100,000 MWCO (UF) or particles size with range of 0.001–0.01 µm [21]. Peak of T2.32 at m/z 257–304 indicated dominant monomers with MW 411.34, 441.39, 397.33, 447.41 and 425.38 Da. and relative intensities 95, 92, 93, 85 and 85%,

Fig 4. (a) Blanched spinach at 15 min, (b) initial fermented spinach (room temperature, 0 day), (c) fermented spinach (room temperature, 5 days), (d) blanched broccoli at 5 min, (e) initial fermented broccoli (room temperature, 0 day), and (f) fermented broccoli (room temperature, 5 days).

respectively. While, peak of T2.82 at m/z 415–470 showed dominant monomers with MW 445.35, 445.15, 445.86, 453.36 and 462.37 Da., respectively with relative intensities 2.41, 2.66, 1.3, 2.13 and 0.92%. Peak of T2.82 was also found monomer as glutamic acid with MW 148.112, 148.29 and 148.57 Da. with relative intensities 0.15, 0.21 and 0.22%, respectively. Table 5 showed predicted suitable monomer with standard folic acid and glutamic acid, in which monomers mentioned before are few monomer from all monomers as folic acid fractionation. Folic acid and their derivatives are known as micronutrient, so that it is only recovered in less content compared to other compounds, such as protein, carbohydrate, fat.

Fermentation process on blanched spinach (Fig. 4a) causes a physical change, especially color in fermented spinach suspension from fresh green (initial fermentation, 0 day) to brownish yellow (5 days of fermentation) followed by fresh aroma like vinegar, as shown in Fig. 4b and 4c, and fermentation process on blanched broccoli (4d), fermented broccoli at both room temperature, and 0 day and 5 days in Fig. 4e and 4f.

CONCLUSION

The experimental activities showed that SUFC mode operated at lower stirrer rotation speed and higher pressure separated folic acid and polyphenol successfully, and separated reducing sugar, total acids and total solids unsuccessfully. Based on selectivity and recovery of folic acids, UF process optimization was achieved under stirrer rotation speed 200 rpm and pressure 40 Psi with generated fermented spinach retentate (concentrate) with compositions of folic acid 42.81 µg/mL, total acids 0.58%, total solids 9.4%, reducing sugar 92.65 mg/mL and total polyphenol 0.087%, and fermented spinach permeate with compositions folic acid 20.39 µg/mL, total acids 0.39%, total solids 8.1%, reducing sugar 159.37 mg/mL and total polyphenol 0.08%. In this optimum process condition, SUFC mode was able to recover folic acid 67.75% from folic acids in retentate and permeate (63.19 µg/mL) with rejection factor 97.27%. Identification on monomer for permeate from the optimum process treatment was resulted folic acid monomer with MW 441.39 and relative intensity 93% at mass spectra T2.32 ranging m/z 257–304, and glutamic acid monomer with MW 148.57 and relative intensity 0.22% at mass spectra T2.82 ranging m/z 415–470. Another dominant monomer was indicated as folic acid fractionation.

The recovery and quality of folic acid can be performed through a series of membrane process (such as microfiltration–ultrafiltration) with selection of kind of membranes which is suitable and appropriate.

REFERENCES

[1] Ural, and Serdar, H., 2008, *Folic Acid and Pregnancy, Kid's Health*, http://kidshealth.org/en/parents/preg-folic-acid.html, Accessed on April 18, 2017, 11.49.

[2] Anonymous, 2012, *Dietary Supplement Fact Sheet: Folate, Office of Dietary Supplements,* National Institutes of Health, http://ods.od.nih.gov/factsheets/folate.asp, Accessed on December 8, 2014.

[3] Jayabalan, R., Subathradevi, P., Marimuthu, S., Sathshkumar, M., and Swaminathan, K., 2008, Changes in free-radical scavenging ability of kombucha tea during fermentation, *Food Chem.*, 109 (1), 227–234.

[4] Jayabalan, R., Malbaša, R.V., Lončar, E.S., Vitas, J.S., and Sathishkumar, M., 2014, A Review on Kombucha tea–microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus, *Compr. Rev. Food Sci. Food Saf.*, 13 (4), 538–550.

[5] de Crécy-Lagard V., El Yacoubi, B., de la Garza, R.D., Noiriel, A., and Hanson, A.D., 2007, Comparative genomics of bacterial and plant folate synthesis and salvage: Predictions and validations, *BMC Genomics*, 8, 245.

[6] Hauser, P.M., and Macreadie, I.G., 2006, Isolation of the *Pneumocystis carinii* dihydrofolate synthase gene and functional complementation in *Saccharomyces cerevisiae*, *FEMS Microbiol. Lett.*, 256 (2), 244–250.

[7] Rezzadori, K., Serpa, L., Penha, F.M., Petrus, R.R., and Petrus, J.C.C., 2014, Crossflow microfiltration of sugarcane juice - effects of processing conditions and juice quality, *Food Sci. Technol.*, 34 (1), 210–217.
[8] Susilowati, A., Aspiyanto and Melanie, H., 2017, Characterization of fermented broccoli (*Brassica oleracea* L.) and spinach (*Amaranthus* sp.) produced using microfiltration membrane as folic acid source for smart food formula, *MATEC Web Conf.*, 101, 01005.
[9] Humpert, D., Ebrahimi, M., and Czermak, P., 2016, Membrane technology for the recovery of lignin: A review, *Membranes*, 6 (3), E42.
[10] Pinto, P.C.R., Mota, I.F., Loureiro, J.M., and Rodrigues, A.E., 2014, Membrane performance and application of ultrafiltration and nanofiltration to ethanol/water extract of *Eucalyptus* bark, *Sep. Purif. Technol.*, 132, 234–243.
[11] Wickramasinghe, S.R., Bower, S.E., Chen, Z., Mukherjee, A., and Husson, S.M., 2009, Relating the pore size distribution of ultrafiltration membranes to dextran rejection, *J. Membr. Sci.*, 340 (1-2), 1–8.
[12] Massé, A., Thi, H.N., Legentilhomme, P., and Jaouen, P., 2011, Dead-end and tangential ultrafiltration of natural salted water: Influence of operating parameters on specific energy consumption, *J. Membr. Sci.*, 380 (1-2), 192–198.
[13] Anonymous, 2008, *Catalogue and Manual of Stirred Ultrafiltration Cells*, Amicon Bioseparations, MILLIPORE, U.S.A.
[14] Anonymous, 1995, *Official Methods of Analysis of AOAC International*, Washington D.C.
[15] Pope, C.G., and Stevens, M.F., 1989, The determination of amino-nitrogen using a copper method, *Biochem. J.*, 33 (7), 1070–1077.
[16] Gani, A., Prasad, K., Ahmad, M., and Gani, A., 2016, Time-dependent extraction kinetics of infused components of different Indian black tea types using UV spectroscopy, *Cogent Food Agric.*, 2 (1), 1–9.
[17] Ruengsitagoon, W., and Hattanat, N., 2012, *Simple spectrophotometric method for determination of folic acid*, The 4th Annual Northeast Pharmacy Research Conference, February 11–12, 2012, Khon Kaen University, Thailand.
[18] Eichhorn, P., and Knepper, T.P., 2001, Electrospray ionization mass spectrometric studies on the amphoteric surfactant cocamidopropylbetaine, *J. Mass Spectrom.*, 36 (6), 677–684.
[19] Susilowati, A., Iskandar, J.M., Melanie, H., Maryati, Y., Lotulung, P.D., and Aryani, D.G., 2015, *Pengembangan konsentrat sayuran hijau dan kacang-kacangan terfermentasi pada jagung (Zea mays L.) pramasak sebagai sumber asam folat untuk formula pangan pintar*, Research Report, Research Center for Chemistry – Indonesian Institute of Sciences.
[20] Aspiyanto, Susilowati, A., Iskandar, J.M., Melanie, H., Maryati, Y., and Lotulung, P.D., 2016, Characteristic of fermented spinach (*Amaranthus* spp.) polyphenol by Kombucha culture for antioxidant compound, *Conf. Proc. Int. Symp. Appl. Chem. 2016 (ISAC 2016)*, Tangerang, October 3–5 2016.
[21] Marchetti, P., Solomon, M.F.J., Szekely, G., and Livingston, A.G., 2014, Molecular separation with organic solvent nanofiltration: A critical review, *Chem. Rev.*, 114 (21), 10735–10806.
[22] Bilad, M.R., 2010, *Teknologi membran untuk aplikasi BRM*, https://roilbilad.wordpress.com/2010/07/22/2-teknologi-membran-untuk-aplikasi-brm/, Accessed on October 25, 2011.
[23] Susilowati, A., Aspiyanto, Melanie, H., and Maryati, Y., 2010, Green tea (*Camellia assamica*) concentrate as a source of L-theanine used in kombucha fermentation for relaxation drink, *Menara Perkebunan*, 78 (2), 75–83.
[24] Susilowati, A., Aspiyanto, Maryati, Y., and Melanie, H., 2016, Characteristic of kombucha tea concentrate purified through stirred microfiltration cell (SMFC) from local green tea (*Camellia* sp.) as functional drink for relaxation, *Jurnal Penelitian Teh dan Kina*, 19 (1), 67–84.
[25] Belitz, H.D., Grosch, W., and Schieberle, P., 2009, *Food Chemistry*, 4th revised and extended ed., Springer-Verlag, Berlin Heidelberg.