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Membrane Performance of Micro- and Ultrafiltration on Folic Acid Separation from Dent Corn (Zea mays var. indentata) Hydrolyzed by Rhizopus oligosporus-C1

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***Abstract*--Performance of microfiltration (MF) and ultrafiltration (UF) membranes to separate of folic acid from corn (Zea mays var. indentata) hydrolysate equipped in stirred filtration cell (SFC) mode were performed as a reference guide toward semi-pilot scale. Separation on hydrolysate suspension of yellow dent corn (HSYCD) and hydrolysate suspension of white dent corn (HSWCD) as a results of hydrolysis of protease enzyme of Rhizopus oligosporus strain-C1 0.025 % and 0.075 % (w/w, dissolved protein) at pH 5 and 30 °C for 24 hours was conducted by MF (0.45 µm) and UF (100000 MWCO) membranes at stirrer rotation speed 400 rpm and trans-membrane pressure (TMP) of 20, 30 and 40 psia for 30 minutes. The experimental result showed that based on optimization of fluxes, the best performances of MF and UF membranes on HSYDC and HSWDC were achieved at TMP 40 psia and gave fluxes of 0.0534 and 0.0508 mL/cm2.min., respectively. In these process conditions, it takes to place an increase of folic acid in concentrates of HSYDC and HSWDC compared with before process (feed). Identification of molecular weight (MW) on folic acid from HSYDC and HSWDC displayed dominant folic acid monomer at T2.7, and T2.4 and T2.89. This matter showed that commodity of yellow dent corn has more potential use as a source of folic acid compared with white dent corn at both similar condition of hydrolysis and separation and purification processes.**

***Keywords*—membrane; folic acid; dent corn (Zea mays var. indentata); retentate; permeate.**

I. INTRODUCTION

A. Background

The use of pressure-based driven membrane system, such as micro- and ultrafiltration in separating corn hydrolysate generated through hydrolysis of corn using crude protease enzyme of Rhizopus oligosporus-C1 is an effort to separate and recover folic acid as a concentrate for fortificant of smart food [1],[2]. Smart food has expressed a form of real food with nutritional value consisted of components contributing smartness, particularly folic acid (vitamin B9) [3]. In MF and UF mode, low pressured solution based on molecular size is forced against a membrane having a certain pores size relating to components molecular weight (MW) separated. The enriching relatively high MW components retained completely on the top membrane surface are called as non-permeable solids retentate (concentrate) based on a sieving mechanism.

Meanwhile, the depleting a pure solvent (water), most ions, salts and low MW components (solutes) passing freely and totally via the membrane is expressed as permeable solutes (permeate) [4]. In applying MF (pore sizes of 0.1 µm – 10.0 µm and their molecular weight cut-off (MWCO) of 300 kDa. – 1,000 kDa) [5] and UF (pore sizes of 0.001 µm – 0.1 µm and their MWCO of 1 kDa. – 300 kDa. [6], whereas folic acid (MW 441 Da.) [7] will penetrate both the MF and UF membranes as permeate. The MF and UF membrane techniques are selected because folic acid is instable, particularly at high temperature. Both MF and UF membranes with the type of fluoro polymer have product specification with a pore size of 0.45 µm and a broad range of typical 1 kDa. – 1,000 kDa., range of pressure 1 – 10 bar and 1 – 10 bar, range of temperature 0 – 60 oC and 0 – 60 oC, and pH range of 1 – 11.5 and 1 – 11, respectively [8].

As a comparison in separating process of corn hydrolysate, use of MF membrane (0.45 µm) can retain and sieve macromolecules > 500.000 MWCO (> 0.1 – 10 µm), such as polysaccharides (8 – 20 µm) on the top membrane surface, meanwhile other compounds in corn hydrolysate with particles size range < 0.45 µm, such as fat (1 – 10 µm), protein (0.04 – 2 µm), pigment of beta-carotene (0.1 – 10 µm), polyphenol, organic acids, amino acids, vitamin (folic acid and other vitamin), and mineral (0.001 – 0.1 µm) passes and penetrates freely through membrane ([9],[10]). Using MF and UF membranes fitted in dead-end Stirred Filtration

Cell to facilitate separation mechanism because direct feed flow is forced against the membrane, in which the only outlet for upstream fluid is through the membrane.

Some solids and the filtered components will accumulate behind on the membrane surface, while water flows through it. Rejected components concentration in bulk solution will become more and higher so that permeate quality will drop on process time [11]. In order to know the characteristic of oligomer produced by hydrolysis process, identification of oligomer by Liquid Chromatography coupled with Mass Spectrometry (LC-MS) can be monitored MW range of folic acid so that domination of monomer in hydrolysate can be known.

By using chromatography technique, mixture of molecular is able to be separated based on difference in migration speed and molecule distribution in stationary phase (adsorbent) and mobile phase (eluent), whereas mass spectrometry will ionize analyte according to principle of Electro Spray Ionization (ESI) to gas phase (fine aerosol) [12]. LC-MS will separate folic acid monomer and identify it according to MW and relative intensity. The difference in both types of membranes is enabled to generate a difference in concentrate and permeate characteristic affected by rotation speed, pressure, and time in SFC, as well. The difference in the feed of nixtamalized corn hydrolysate with viscosity, turbidity, and composition of nutrition (folic acid) enable to be achieved concentrate and permeate with the suitable and appropriate characteristic as fortificant.

B. Objective

The experimental activity aimed to know performances of MF and UF membranes equipped in SFC via different TMP at both fixed stirrer rotation speed, temperature and time in separating folic acid on performance and characteristic of the folic acid monomer as a concentrate of yellow dent corn and white dent corn for fortificant of smart food.

II. MATERIAL AND METHOD

A. Materials and Equipment

Main materials used in this experimental activity were dry yellow dent corn and dry white dent corn from Tangerang and Rhizopus oligosporus-C1 fungi (0.025 % and 0.075 % (w/w, dissolved protein) (Research Center for Chemistry – LIPI). The chemical reagents for preparation and analysis purposes were Ca(OH)2 (E.Merck), methanol (E.Merck), hydrochloric acid (E.Merck), sodium nitrite (E.Merck), sulfamic acid (Sigma-Aldrich), 3-aminophenol (Sigma-Aldrich), standard folic acid (Sigma-Aldrich), and standard glutamic acid (Sigma-Aldrich). All of the chemical reagents employed comprise analytical grade quality and the highest purity available obtained from commercial sources and used without further purification. Commercial MF membrane (Fluoro polymer, pore size 0.45 µm, DSS, Denmark) and UF membrane (Fluoro polymer, 100000 MWCO, Alfa Laval, Denmark) were used. Equipment used in this experimental works were nixtamalization process system, grinder (local), the sieve of 60 mesh (Retsch, Germany) and hydrolysis system (Shaker batch, CertomatR WR), Dead-End Stirred Filtration Cell (SFC) (Model 8200, Amicon Bioseparation, MILLIPORE, U.S.A.) [13]. The main analysis instrument was Liquid Chromatography-Mass Spectrometry (LC-MS) (Mariner Biospectrometry) equipped with LC (Hitachi L 6200).

B. Experimental design

Experimental works were performed by passing hydrolysate suspension of yellow dent corn (HSYDC) and white dent corn (HSWDC) through MF membrane (0.45 µm), and UF membrane (100000 MWCO) fitted in SFC at stirrer speed 400 rpm, TMP 20, 30 and 40 psi, and room temperature for 30 minutes. Measuring was performed on flux from some volume passing via a unit of membrane surface area normal to the thickness direction per unit time [13]. The analysis was conducted on feed, retentate and permeate covering total solids (Gravimetric method) [14] and folic acid (UV-vis Spectrophotometer) [15]. Identification on folic acid and glutamic acid were done via Liquid Chromatography-Mass Spectrometry (LC-MS) (Mariner Biospectrometry) equipped with LC (Hitachi L 6200) [16]. Process and analysis were performed in duplicate. Data were processed in this description based on the result of the average analysis.

C. Procedure steps

1) Hydrolysis process and separation of the component through membranes: Hydrolysis process of corn was started by treating nixtamalization on corn. A number of dry yellow dent corn and dry white dent corn was washed, steeped in water (1 part of corn/4 parts of water) for 18 hours, added solution of $Ca(OH)_2$ (20 %, w/w corn dissolved protein) and solution of $Ca(OH)_2$ (30 %, w/w corn dissolved protein), boiled at 90 °C for 60 minutes and 90 °C for 30 minutes, respectively, allowed, and blended or grinded. Nixtamalized corn (nixtamal) was further hydrolyzed by R. oligosporus C_1 at concentrations of 0.025 % and 0.075 % (w/w, dissolved protein) at 37 °C and pH 5 for 24 hours [17], and sieved through a 80 mesh to obtain a filtrate with uniform particle size introduced as feed-in separation and/or recovery of folic acid. The UF membrane discs having a 100.000 MWCO with the glossy skin side toward a solution was placed into the SFC mode connected to a nitrogen gas cylinder as driving force of fluid feed. Before use, membrane fitted in SFC (180 mL capacity) was rinsed with pure water. Each membrane was first compacted by flowing pure-water through the membranes at 10 psi for 5 minutes. Compaction ensured that all solvents used during the manufacturing were removed from the membrane's surface and pores. Further, pure water in the feed cell was drained and replaced with HSYDC. SFC was operated at stirrer rotation speed of 400 rpm and TMP of 20 psi for 30 minutes. Permeate passing via pores of membrane was collected and measured its volume permeate flow rate in the time interval. Besides, particles retained at top membrane surface was expressed as retentate. Further, components in permeate and retentate were analyzed. A similar procedure of separation according to experimental design was performed on HSWDC and MF membrane (0.45 µm).

2) Identification of folic acid through LC-MS: Samples of permeate as a result of the separation of HSYDC from the best treatment performed using MF and UF membranes were standard folic acid and standard glutamic acid. Oligomers

were analyzed via LC-MS instrument using Mariner Biospectrometry. LC system was integrated with Q-tof mass spectrometer via Electrospray Ionization (ESI), in which scan mode is conducted in the range of m/z 100 – 1200 at 140 °C. LC (Hitachi L 6200) uses a C18-18 RP (5 µm particle size, 15 cm x 1 mm i.d.) column from Supelco (Bellefonte, PA). Type of solvent used is methanol at a flow rate of 0.1 mL/min. and the injection volume was 2 uL.

3) Analysis of folic acid: Analysis of folic acid was performed by using spectrophotometry according to diazotization reaction of N-(4-Aminobenzoyl)-L-glutamic acid diethyl ester generated after reduction reaction between folic acid and 3-aminophenol to form a yellow-orange complex. One mL of sample of standard folic acid was added by 1 mL of 4 M hydrochloric acid, 1 mL of sodium nitrite 1 % (w/v), 1 mL of sulfamic acid 1 % (w/v) and 1 mL of 3-aminophenol 1 % (w/v) and vortexed to form yellow-orange complex. Absorbance was further monitored by UV-vis spectrophotometer at the wavelength of 460 nm**.**

III. Results and Discussion

A. Characteristic of HSYDC and HSWDC

HSYDC and HSWDC were turbid and yellowish white suspension relating with the initial characteristic of corn materials. Their compositions on original material and nixtamal of yellow dent corn and white dent corn are summarized in Table 1 and 2. The composition of the primary material of HSWDC showed higher concentrations of folic acid (211.24 ug/mL) and total solids (93.86 %) when compared to folic acid (159.7 ug/mL) and total solids (87.52 %) in HSYDC. The difference in these concentrations was not only caused by a variety of corns but also by treating post-harvest. Hydrolysis process of both types of corn using protease enzyme of Rhizopus oligosporus-C1 fungi caused its occurrence of increase of folic acid and decrease of total solids. This matter is caused by the effect of hydrolysis, in which the activity of protease enzyme will degrade corn protein to amino acids. Folic acid is a derivative of protein as glutamic acid consisting of pteridine heterocyclic, para-aminobenzoic acid (PABA) and N-Pteroyl-L-glutamic acid due to an increase of concentration as dissolved protein [18]. Protease enzyme of Rhizopus oligosporus-C1 fungi has the best activity in the range of 30 – 37 °C, and pH 5 suitable with the type of substrate.

TABLE I
THE COMPOSITION OF THE RAW MATERIAL

The composition of raw material	**Components**	**Corn**	**Hydrolysate**	**Feed***
Yellow dent corn	Folic acid (µg/mL)	159.70	298.84	207.59
	Total solids (%)	87.52	19.27	5.32
White dent corn	Folic acid (µg/mL)	211.24	195.17	163.85
	Total solids (%)	93.86	20.47	4.38

Legend : *sieved through an 80 mesh

Table 1 above indicates the presence of high content of protein in corn (7 – 10 %) becomes a reference guide in this selected cereal, besides the high content of folic acid (26 µg) and its potential use as a source of energy. This process drops total solids content, as well caused by degrading components in corn due to gelatinization during hydrolysis. Declining folic acid and total solids also seem on filtrate passing through an 80 mesh sieve in order to achieve membrane performance in separating folic acid.

B. Effect of separation process condition on membrane performance

1) Fluxes

MF and UF are very useful non-thermal unit operations in chemical engineering to separate and recover some valuable, target and certain components or compounds from food processing and fermentation broths. For all MF and UF modes, the flow rate of permeate, abbreviated to flux and rejection of solutes are the main factors used to evaluate the performance of membranes. Flux, affecting the viability of many membrane separation processes, is usually presented regarding the amount of permeate volume passing through per unit of membrane surface area in an time period. Factors affecting the flux are the nature and MWCO of the membrane, the nature and concentration of the solutes in the process solution and viscosity, pressure across the membrane or trans-membrane pressure (TMP), and flow rate, which affect turbulence and temperature. Other factors, such as solution pH and osmotic pressure may influence the flux, as well. In MF and UF, there is little osmotic pressure difference over the membrane as the low molecular weight (MW) components are almost freely permeating. Each factor will contribute a different effect on the flux [19]. Separation and/or recovery process on folic acid in HSYDC and HSWDC gave fluxes becoming more and more increase relating with increasing TMP, as showed in Figure 1. The flux histories of UF membrane of HSYDC at TMPs of 20, 30 and 40 psia indicated that flux raise gradually from 0.0438, 0.0478 to 0.0508 mL/cm2.min. and is much higher than that UF membrane of HSWDC from 0.0361, 0.0383 to 0.0431 mL/cm2.min.

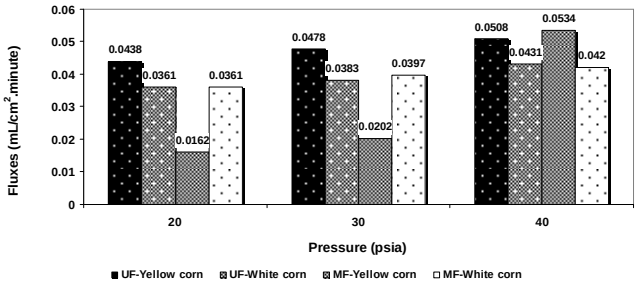

Fig. 1. Effect of TMP on fluxes at the type of membranes, and HSYDC and HSWDC.

This result reflects the viscosity of HSYDC is lower compared with HSWDC. Besides, the number of components in the both HSYDC and HSWDC used as feed is insignificant. This matter will influence the direct flow of HSYDC and HSWDC to pass through pores of the membrane. Meanwhile, an increase of TMP of 20, 30 and 40 psia at MF membrane of HSWDC raised flux gradually from

0.0361, 0.0397 to 0.042 mL/cm2.min. and higher when compared to MF membrane of HSYDC from 0.0162 mL/cm2.min to 0.0202 mL/cm2.min (gradually) to 0.0534 mL/cm2.min. (sharply). The passage of water mass through the membrane pores are caused by a driving force and depends on a membrane porosity, membrane material, hydrophilicity, thickness, roughness, charge, etc. Besides, the smaller (tighter) MF membrane pore sizes occasionally overlap the larger (more open) UF pores. However, UF pores are generally smaller than MF pores. Separation and/or recovery of solids from HSYDC and HSWDC by UF membrane and MF membrane have been conducted in this experimental activity. Recovery of solids demonstrated how much fraction of solute components, originally present in the feed or concentrate, have been separated and/or removed by the membrane. Although folic acid is small molecules (MW 441), it was rejected by the UF membrane and MF membrane. The use of membrane filtration in HSYDC and HSWDC treatments can basically be categorized into recovery of large particle-sized solids and recovery of folic acid for fortifying smart foods.

2) Folic acid

Effect of TMP on folic acid content in retentate and permeate from separation of folic acid in HSYDC via MF and UF membranes, and HSWDC via MF and UF membranes are shown in Figures 2 and 3. Raise of TMP of 20, 30 and 40 psia at MF membrane of HSYDC fluctuated folic acid concentration in retentate rather dramatically from 72.48 µg/mL to 37.15 µg/mL and increased rather sharply from 37.15 µg/mL to 55.29 µg/mL, and lower when compared to permeate of HSYDC which tends to increase 76.05, 83.09 and 84.82 µg/mL. This matter is caused by larger pores size of MF membrane so that convective transport of folic acid component is relative high collected in permeate side.

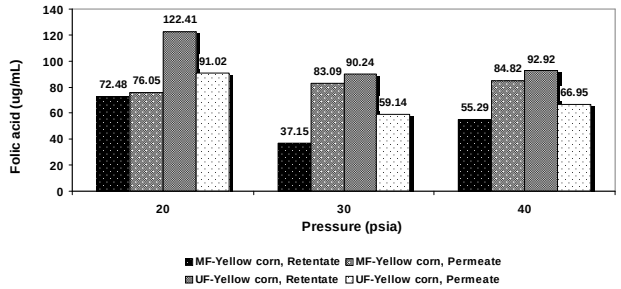

Fig 2. Effect of TMP on folic acid content in retentate and permeate from separation of folic acid in HSYDC via MF and UF membranes.

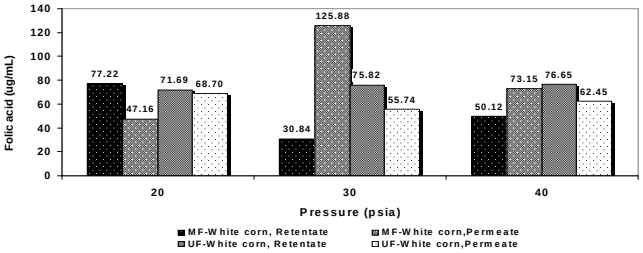

Fig 3. Effect of TMP on folic acid content in retentate and permeate from separation of folic acid in HSWDC via MF and UF membranes.

Increase of TMP of 20, 30 and 40 psia at UF membrane technique of HSYDC declined dramatically folic acid concentration in retentate started from 122.41 µg/mL to 90.24 µg/mL and increased gradually to 92.92 µg/mL, meanwhile with whatever the TMP (20, 30, 40 psia) dropped sharply folic acid concentration in permeate originated from 91.02 µg/mL to 59.14 µg/mL and increased slightly to 66.95 µg/mL. Folic acid concentration in retentate of HSYDC is higher than that in permeate of HSYDC. This difference in concentration is as a result of separation, discrimination and/or recovery between molecules primarily on the basis of size and with different physical characteristics. It had been observed (Figure 2b) that as the TMP increases 20, 30 and 40 psia at MF membrane process of HSWDC, folic acid concentration in retentate decreases rather sharply from 77.22 µg/mL to 30.84 µg/mL and increases rather slighly to 50.12 µg/mL, meanwhile folic acid concentration in permeate increases sharply started from 47.16 µg/mL to 125.88 µg/mL and declines to approximately 73.55 µg/mL. At TMP of 20 psia, folic acid concentration in retentate is higher than that in permeate. On the other hand, at TMP 30 psia and 40 psia, folic acid concentration in permeates are higher that that in retentates. This indicated that by using higher TMP (30 psia and 40 psia) will force folic acid molecules to pass freely membrane collected in permeates. By increasing TMP of 20, 30 and 40 psia at UF membrane on HSWDC, the folic acid concentrations in retentate raised slightly 71.69, 75.82 and 76.65 µg/mL, and in permeate dropped gradually from 68.70 µg/mL to 55.74 µg/mL and started to increase slightly to 62.45 µg/mL. Folic acid concentration in retentate was higher than that in permeate. It had been known that pores size of UF membrane is smaller than that MF membrane so that feed fluid forcing component through UF membrane gives minimal concentration of component in UF permeate.

3) Total solids

Relationship between type of membranes and TMP in in SFC on separation and/or recovery of total solids in (a) HSYDC and (b) HSWDC were shown in Figures 3a and 3b. By means of MF membrane of HSYDC operated at TMP of 20, 30 and 40 psia, the total solid concentration in retentate increased slightly 3.76, 3.95 and 5.09 %, respectively. Meanwhile, the total solid concentration in permeate is stable from 0.93 % to 0.93 % or independent of TMP and decreased slightly from 0.93 % to 0.58 %, respectively. As the TMP increased (from 20, 30 to 40 psia), the total solid concentration in UF membrane of HSYDC increased slightly 4.61, 7.01 and 9.82 %, respectively. Meanwhile, the total solid concentration in the permeate increased slightly from 0.98 % to 1.03 % and began to drop rather sharply to 0.76 %, respectively. By increasing TMP of 20, 30 and 40 psia at MF membrane technique of HSWDC gave a fluctuation concentration result of total solids in retentate started to drop slightly from 5.71 % to 5.45 % and raised from 5.45 % to 8.33 %, respectively. Whereas, total solids concentration in permeate tends to decline gradually from 1.78 % to 1.70 % and stable from 1.70 % to 1.70 % or independent of TMP, respectively. By using UF membrane mode of HSWDC, an increase in TMP (20, 30 and 40 psia) raised gradually total solids concentration in retentate initiated from 13.88 % to 14.76 % and declined from 14.76 % to 14.50 %. Whereas, total solids concentration in permeate showed a gradually

raise from 1.18 %, 1.44 % to approximately 1.52 %, respectively. However, this UF membrane mode can separate total solids successfully and gives results of 91.5, 90.2 and 89.5 %, respectively.

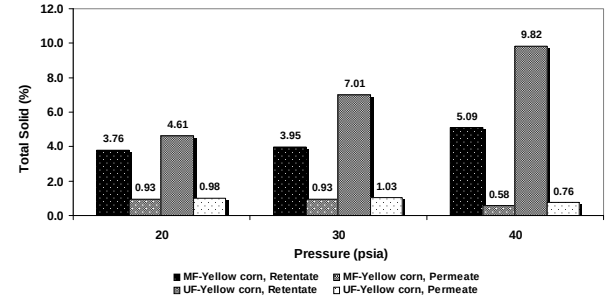

Fig. 4. Effect of TMP on total solids content in the retentate and permeate from the separation of total solids in HSYDC via MF and UF membranes.

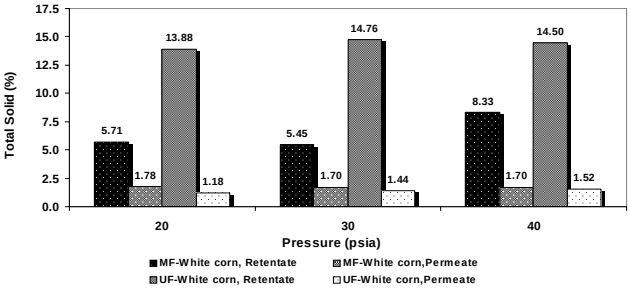

Fig. 5. Effect of TMP on total solids content in the retentate and permeate from the separation of total solids in HSWDC via MF and UF membranes.

From the analyse result of performance effect on both type of membranes fitted in dead-end SFC mode at stirrer speed of 400 rpm, and TMP of 20, 30 and 40 psia for 30 minutes and based on permeate fluxes, optimization conditions were achieved by UF membrane at TMP of 40 psia and MF membrane at TMP of 40 psia using HSYDC and gave permeate fluxes of 0.0508 and 0.0534 mL/cm2. Minute, respectively. In other words, separation and recovery folic acid in hydrolysate suspension of dent corn using MF membrane gave still the best permeate flux compared to the other treatments. Figure 6 displayed feed of HSYDC (a) introduced to UF mode, retentate (b) and permeate (c), and feed of HSYDC (d) introduced to MF mode, retentate (e) and permeate (f) as a result of separation and/or recovery folic acid through UF and MF membranes, respectively.

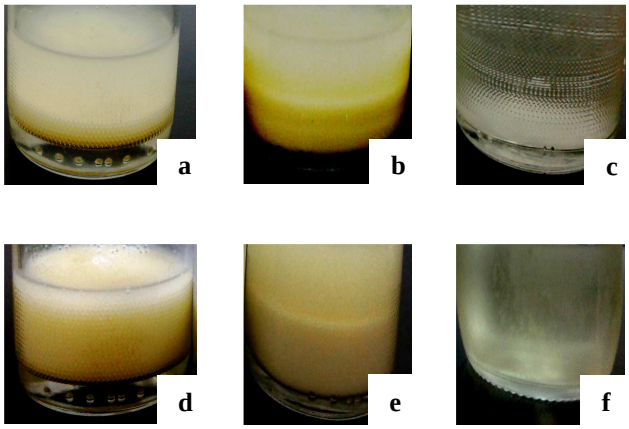

Fig. 6. (a) Feed of HSYDC for UF mode, (b) retentate and (c) permeate, and (d) feed of HSYDC for MF mode (e) retentate and (c) permeate.

C. Identification of folic acid on hydrolyzed corn

1) Standard folic acid and standard glutamic acid: Identification on monomer of hydrolyzed corn was conducted on folic acid and glutamic acid as a part of folic acid. Identification result on standard glutamic was reached 1 peak (T3.0) ranging in retention time 0 – 10 minutes and relative intensity of 100 %, in which mass spectra m/z 111 – 784 from T3.0 displayed compound domination with MW 148.1479 Da. (100 %), as shown in Figures 7, 8, 9 and 10, respectively. Glutamic acid is a combination of pteridine heterocyclic, para-aminobenzoate acid (PABA) and glutamic acid [20]. On standard folic acid was achieved one peak (T1.7) with retention time 0 – 10 minutes and relative intensity 100 %, in which mass spectra m/z 425 – 498 from T1.8 demonstrated compound domination with MW 442.76 Da. (100 %), 443.7 Da. (25 %), 441.48 Da. (5 %), as shown in Figures 6c and 6d. It had been known that due to folic acid MW of 441 Da. [18]. LC-MS method (a type of solvent, injection concentration, flow rate) enabled its occurrence of folic acid degradation [16]. Via LC-MS method had been known that a compound indicated a difference in MW, in which its possibility is as M^+, M^+ Na^+, $2M^{++}$ or $2M^+$, Na^+. This matter is caused by its presence of ionization as a consequence of sensitivity of LC-MS instrument relating to eluent used.

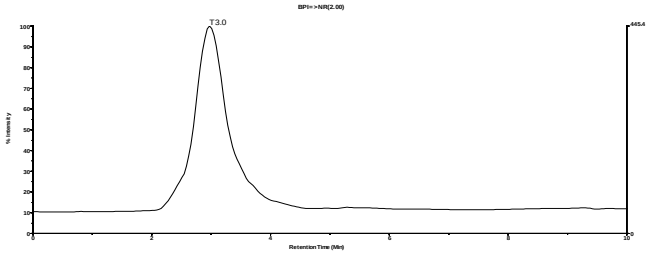

Fig. 7. Chromatogram of standard glutamic acid with retention time 3.0.

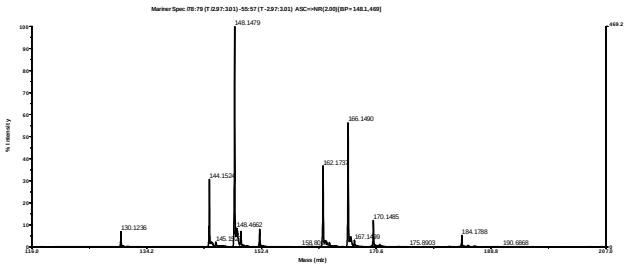

Fig. 8. Mass spectra at T3.0 in standard glutamic acid.

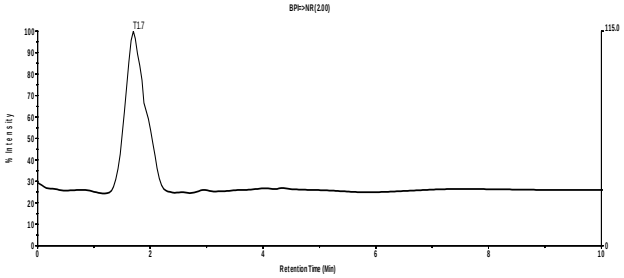

Fig. 9. Chromatogram of standard folic acid with retention time 1.7.

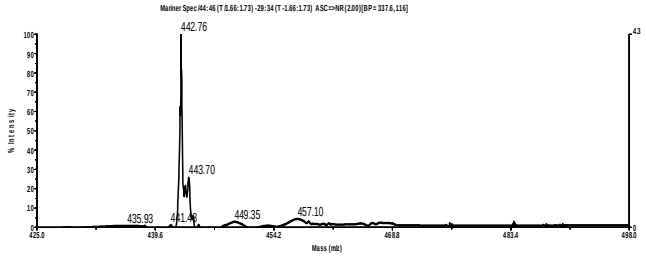

Fig. 10. Mass spectra range at T 1.7 in standard folic acid.

2) Hydrolysate suspension of HSYDC from UF membrane: Identification result of HSYDC based on the best flux value by using UF membrane (stirrer speed 400 rpm and TMP 40 psia) was achieved chromatogram with 2 peaks (T1.53 and T2.4) at retention time 0 – 10 minutes (Figure 11). Mass spectra range m/z 99 – 1200 at T1.53 (Figure 12) did not show glutamic acid monomer, although it was obtained 3 glutamic acid monomers with MW of 148.34, 148.49 and 148.78 Da. with relative intensities of 0.74, 0.64 and 0.35 %, respectively. Glutamic acid monomer starts to seem at T1.53 with mass spectra range of m/z 148.03 – 149.04 dominated by monomer at MW 148.49 Da. (M^+) With relative intensity 100 % (Figure 13). The similar matter seemed on folic acid monomer which was not monitored at T1.53 with mass spectra range m/z 99 – 1200, although it had been monitored 1 folic acid monomer with MW range of 442.78 Da. and relative intensity range of 0.96 %, however m/z 440.09 – 443.15 was dominated by monomer of MW 442.6 Da. (M^+) with relative intensity of 100 % (Figure 14). With mass spectra range m/z 147.25 – 149.40 at T2.4 was reached glutamic acid monomer at MW 148.32 Da. with relative intensity 100 %, while folic acid seemed at mass spectra range m/z 440.56 – 443.59 with MW 441.24 Da. and relative intensity 100 % (Figures 15 and 16).

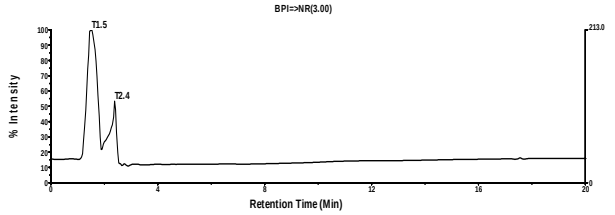

Fig. 11. Chromatogram of extract of HSYDC separated by UF membrane at T1.53 and T2.4 with retention time 0 – 10 minutes.

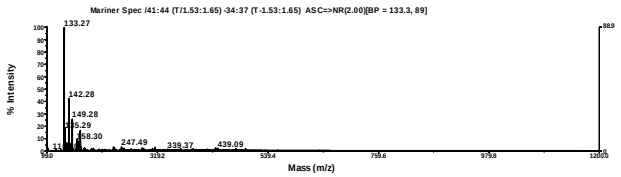

Fig. 12. Mass spectra range m/z 99 – 1200 of HSYDC extract separated by UF membrane.

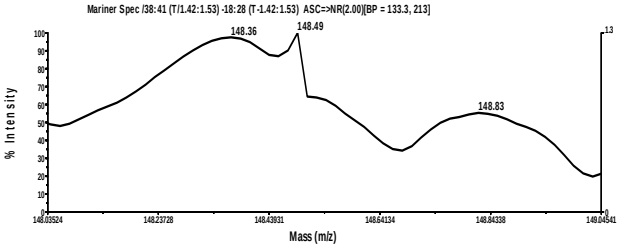

Fig. 13. Mass spectra for glutamic acid monomer of HSYDC extract separated by UF membrane at T1.53.

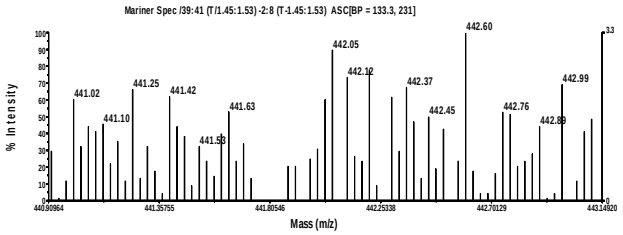

Fig. 14. Mass spectra for folic acid monomer of HSYDC extract separated by UF membrane at T1.53.

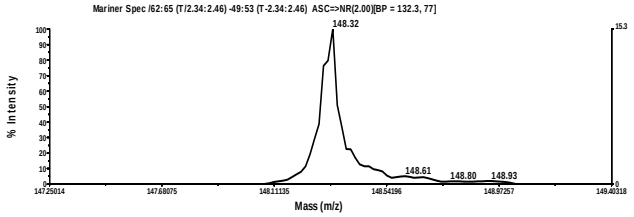

Fig. 15. Mass spectra for glutamic acid monomer of HSYDC extract separated by UF membrane at T2.4.

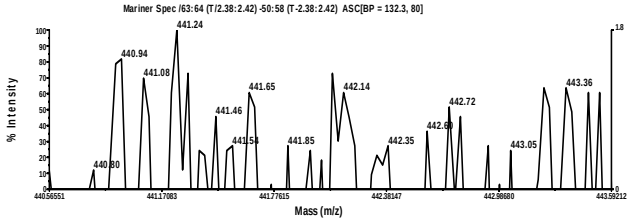

Fig. 16. Mass spectra for folic acid monomer of HSYDC extract separated by UF membrane at T2.4.

3) Hydrolysate suspension of HSYDC from MF membrane: Identification result of HSYDC based on the best flux value by using MF membrane (stirrer speed 400 rpm and TMP 40 psia) was obtained chromatogram with 2 peaks (T2.9 and T4.1) at retention time 0 – 10 minutes (Figure 17). Mass spectra range m/z 99 – 1200 at T2.9 (Figure 18) did not show glutamic acid monomer, despite it had been achieved 1 monomer of glutamic acid at MW of 148.49 Da. with relative intensity 0.55 %. The monomer of glutamic acid was immediately monitored at T1.53 with mass spectra range m/z 144 – 156.41 dominated by monomer at MW of 148.29 Da. (M^+) with relative intensity 100 % (Figure 19). The same matter did not monitor for the monomer of folic acid with mass spectra range m/z 99 – 1200 at T2.9, however it was monitored with mass spectra range m/z 441 – 443 dominated by monomer at MW of 442.96 Da. (M^+) with relative intensity 100 % (Figure 20). Mass spectra range m/z 146 – 150 at T4.1 was obtained monomer of glutamic acid at MW of 148.29 Da. with relative intensity 100 %, meanwhile monomer of folic acid monitored at mass spectra range m/z 441 – 443 had MW of 441.91 Da. with relative intensity 100 % (Figures 21 and 22).

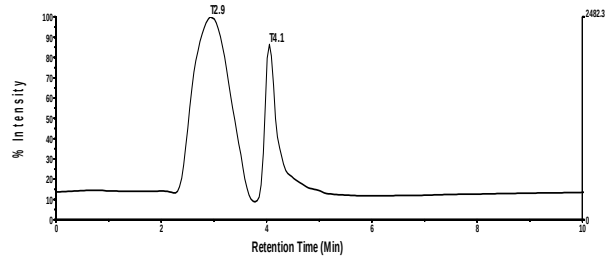

Fig. 17. Chromatogram of an extract of HSYDC separated by MF membrane at T2.9 and T4.1 with retention time 0 – 10 minutes.

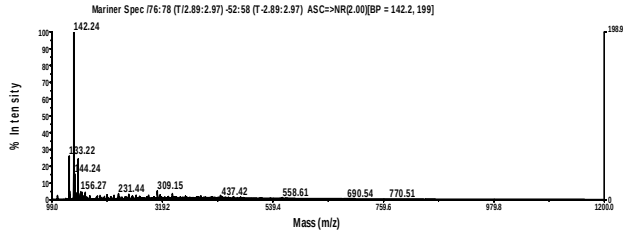

Fig. 18. Mass spectra range m/z 99 – 1200 of HSYDC extract separated by MF membrane.

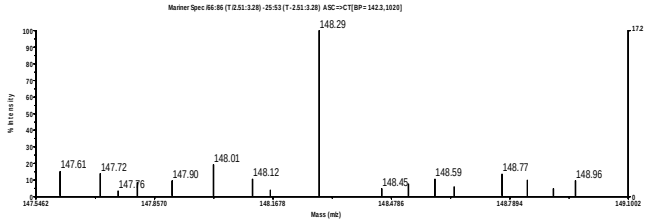

Fig. 19. Mass spectra for the glutamic acid monomer of HSYDC extract separated by MF membrane at T2.9.

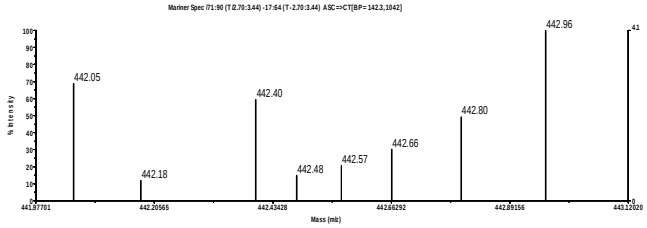

Fig. 20. Mass spectra for the folic acid monomer of HSYDC extract separated by MF membrane at T2.9.

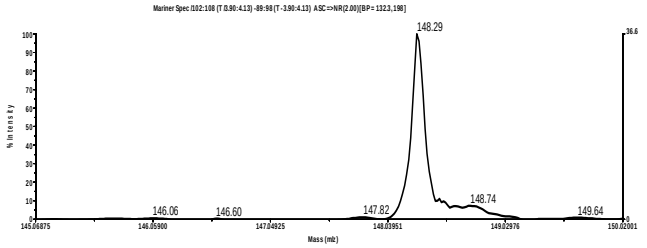

Fig. 21. Mass spectra for the glutamic acid monomer of HSYDC extract separated by MF membrane at T4.1.

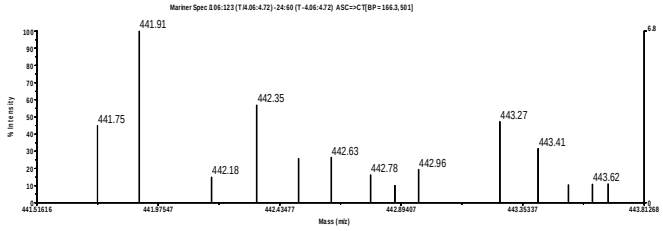

Fig. 22. Mass spectra for the folic acid monomer of HSYDC extract separated by MF membrane at T4.1.

IV. Conclusion

Both MF and UF processes can effectively be used to separate and recover folic acid as a target and desired component from HSYDC and HSWDC. Based on one of the most important membrane performances, the highest permeate flux was achieved by MF membrane (0.45 µm) operated at 400 rpm and TMP 40 psia using HSYDC due to larger pores size compared with UF membrane and gave 0.0534 mL/cm2.min. Meanwhile, based on other one of the most important membrane performances, the highest recovery of folic acid from HSYDC feed or retentate was obtained via UF membrane operated at 400 rpm and TMP 40 psia using HSYDC and gave 92.92 µg/mL. The UF membrane has a better performance compared to the MF membrane. To recovery target and desired components from HSYDC maximally, such as folic acid (MW 441), HSYDC containing folic acid was filtered firstly using the membrane of largest pore size (MF membrane) followed by concentration and recovery of by UF membrane.

The conclusion for selecting a proper membrane for downstream processing of agricultural products-based hydrolysis and fermentation broth depends on the average size of the desired components, and on the reusability of the membrane and broth for further fermentation steps. Based on permeate flux, identification on the monomer in HSYDC from the optimum condition of UF membrane showed recoveries of a monomer of glutamic acid with MWs of 148.49 and 148.32 Da. and relative intensities of 100 % and 100 %, respectively, and a monomer of folic acids with MW of 442.6 Da. and 441.24 Da. Meanwhile, on HSYDC introduced to MF membrane using fitting in SFC was recovered monomer of glutamic acid with MW of 148.29 Da. and relative intensity of 100 %, and a monomer of folic acid with MWs of 442.98 Da. and 441.91 Da. and relative intensities of 100 % and 100 % relating with the best mass spectra.

References

[1] R.V. Reis and A.L. Zydney, "Bioprocess membrane technology," *Journal of Membrane Science.* 297, pp.16.2007.

[2] B. Bhayani, I.R. Yasarla and B.V. Ramarao, "Membrane filtration of lignocellulosic hydrolysates: Comparison of crossflow and dead-end microfiltration," *Center for Energy Initiatives (CEI),* American Institute of Chemical Engineers (AlChE). October 18, 2011.

[3] FDA News Release, FDA approves folic acid fortification of corn masa flour. April 14, 2016.

[4] J.S. Phale and Y.J. Chendake, "Polysulfone based Ultrafiltration Membrane Preparation by Phase Inversion: Parameter Optimization," *International Journal of Science and Research (IJSR),* June 2016.

[5] I. Ahmed, "High-performance UF polyester - polysulfone membrane," [Ph.D. Thesis]. University Technology Malaysia, Malaysia. 2009.

[6] L.L. Ngoc and S.P. Nunes, "Materials and membrane technologies for water and energy sustainability," *Journal of Sustainable Materials and Technologies,* 7, pp.5. 2016.

[7] NIST. National Institute of Standards and Technology (NIST) Chemistry WebBook, SRD 69, U.S.A. 2017.

[8] O.J. Olsen, "Operating Manual of DSS LabUnit M20," *Danish Separation Systems AS,* DK-4900 Nakskov, Denmark. January 2000.

[9] A.L. Zydney, *High-Performance Ultrafiltration Membranes: Pore Geometry and Charge Effects,* in S.T. Oyama and S.M. Stagg-Williams (Eds.). Membrane Science and Technology, Elsevier, Amsterdam, Vol. 14, pp.333. 2011.

[10] P. Kumar, N. Sharma, R. Ranjan, S. Kumar, Z.F. Bhat, and D.K. Jeong, "Perspective of Membrane Technology in Dairy Industry: A Review," *Asian-Australasian Journal of Animal Sciences (AJAS)*, 26 (9), pp.1347. 2013. DOI: http://dx.doi.org/10. 5713/ajas.2013.13082.

[11] W.J. Koros, Y.H. Ma and T. Shimidzu, T. "Terminology for membranes and membrane process (IUPAC Recommendations 1996)," *Journal of Membrane Science*, 120, pp.149. 1996.

[12] D. Onggo, "General Principles in Electrospray Mass Spectrometry: A New Technique in Mass Spectral Analysis," *Journal of Mass Spectrum*, Vol. 3, No. 2, pp.115. Accessed on February 3, 2013.

[13] MILLIPORE, "Catalogue and Product Information of Stirred Ultrafiltration Cell," *Amicon Bioseparation, MILLIPORE*, Bedford, U. S. A. www.millipore.com. 2008.

[14] A.O.A.C., "Official Methods of Analysis," *Association of Official Analytical Chemists International*, 20th. Edition, Washington D. C. 2016.

[15] W. Ruengsitagoon and N. Hattanat, *Simple spectrophotometric method for determination of folic acid. The 4th Annual Northeast Pharmacy Research Conference*, Thailand. 2012.

[16] P. Eichhorn and T.P. Knepper, "Electrospray ionization mass spectrometric studies on the amphoteric surfactant cocamidopropylbetaine," *Journal of Mass Spectrum*, 36 (6), pp.677 – 84. June 2001. DOI: 10.1002/jms.170.

[17] A. Susilowati, Y.M. Iskandar, H. Melanie, Y. Maryati, P.D. Lotulung and D.G. Aryani, "Pengembangan konsentrat sayuran hijau dan kacang-kacangan terfermentasi pada jagung (*Zea mays* L.) pramasak sebagai sumber asam folat untuk formula pangan pintar," *Laporan Hasil Penelitian, Program Tematik, Kedeputian IPT*, Pusat Penelitian Kimia – LIPI, Kawasan PUSPIPTEK, Serpong, Tangerang Selatan. 2016.

[18] N.A. Rodríguez-Martínez, M.G. Salazar-García, B.R. Wong, A.R. Islas-Rubio, L.C. Platt-Lucero, Morales-Rosas, R. Marquez-Melendez and F. Martínez-Bustos, "Effect of Malting and Nixtamalization Processes on the Physicochemical Properties of Instant Extruded Corn Flour and Tortilla Quality," *Plant Foods Hum. Nutr*. 70, pp.275. Published online: 10 June 2015. Springer Science+Business Media New York. DOI : 10.1007/s 11130-015-0490-9. CrossMark.

[19] A.S. Grandison and M.J. Lewis, *Separation Processes in the Food and Biotechnology Industries: Principles and Applications.* Technomic Publishing Company Inc., Lancaster, Pennsylvania, U.S.A. First publication. 1996.

[20] D. Mancinelli and C. Hallé, "Nano-Filtration and Ultra-Filtration Ceramic Membranes for Food Processing: A Mini Review," *Journal of Membrane Science and Technology*, Vol. 5, Issue 2. 2015. http://dx.doi.org/10. 4172/2155-9589.1000140.