

## Cross Flow Microfiltration System in Separating Fermented Nixtamal Corn for Preparation of Natural Folic Acid

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**Abstract**—A purification process of biomass of nixtamal yellow corn fermented by *Rhizopus oligosporus* strain C1 (FNC-A) and *Rhizopus* sp (FNC-B) was performed using a microfiltration (MF) membrane with pore size 0.15  $\mu\text{m}$  installed in a crossflow microfiltration (CFMF) system to obtain retentate and permeate fractions as natural folic acid preparations. CFMF process was conducted at room temperature with a pump motor frequency (PMF) of 10 Hz (flow rate of 3.5 L/min) and a transmembrane pressure (TMP) of 4 bar for 0, 15, 30, 45, 60, 75, and 90 minutes. Based on optimum folic acid, the results showed that the best treatments of FNC-B and FNC-B were achieved at 90 min, resulting in a folic acid increase in retentate of FNC-A and FNC-B of 72.61 and 95.26%, reducing sugar of 20.41 and 170.93% (1.7-folds), total sugars of 155.51% (1.55-folds) and 426.76% (4.27-folds), and dissolved protein of 55.33 and 39.20%, and decrease in total solids of 88.91 and 91.64%, respectively, compared to initial biomass of FNC-A and FNC-B. The MFCF system effectively separated folic acid in retentates of FNC-A (33.77%) and FNC-B (95.27%) at the optimal condition. Folic acid monomers predominated the characteristics of FNC-A and FNC-B in optimum conditions with molecular weights of 442.10 and 442.18 Dalton, the average particle size of 38.31  $\mu\text{m}$  and 37.97  $\mu\text{m}$ , and distribution of particles at 10, 50, and 90% from the particle size 10.31, 26.32 and 81.55  $\mu\text{m}$ , and 10.37, 28.04 and 76.09  $\mu\text{m}$ , respectively.

**Keywords**— Folic acid; corn; *Rhizopus oligosporus* strain C1; *Rhizopus* sp.; CFMF

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### I. INTRODUCTION

The fermentation process on yellow corn (*Zea mays* var. *indentata*) is carried out to produce natural folic acid by a series of nixtamalization, fermentation, and purification processes of biomass through the CFMF membrane process. Folic acid ( $\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6$ ) has a chemical structure arranged on a pteridine ring, para-Amino Benzoic Acid (pABA) ring and L-glutamic acid [1], [2]. It is a key element in an infant's growth in the womb and has a role in synthesizing Deoxyribonucleic acid (DNA) [3]. Folic acid deficiency can cause congenital disabilities, especially Neural Tube Defects (NTDs) [4]. Folic acid consumption increases when it increases and forms cells, like during pregnancy and premature babies [5, 6]. For a baby, the recommended daily intake of folic acid is about 3.1  $\mu\text{g}$  per kilogram of body weight, or 400  $\mu\text{g}$  per day; for pregnant women and lactating mothers, the recommended daily intake is around 0.4  $\mu\text{g}$ , or 400–600  $\mu\text{g}$  per day [2].

Fermentation of nixtamal corn by *Rhizopus oligosporus* strain C1 and *Rhizopus* sp. (mixture of several species of *Rhizopus*) through a series of steps, such as nixtamalization process by adding  $\text{Ca}(\text{OH})_2$  solution before performing inoculation of fungi, fermentation process for 24–48 hours at room temperature, which is produced mass with the growth of mycelia on the whole surface of nixtamal corn. The nixtamalization process is conducted to increase folic acid, which is influenced by the maize type, cooking time, and  $\text{Ca}(\text{OH})_2$  solution concentration [7]. *Rhizopus oligosporus* strain C1 and *Rhizopus* sp. are single fungi, and the mixture of fungi has a role in degrading the protein of corn to folic acid. These fungi, like other microbes (bacteria, yeast), may be generated by the endogenous biosynthesis of folates in amino acids (methionine, adenine, histidine) to produce folic acid derivatives, including dihydrofolate and polyglutamate folate [8]. This biosynthesis is present in both plants and microbes.

The separation process of folic acid from Fermented Nixtamal Corn (FNC) through a crossflow microfiltration

(CFMF) process system is applied because folic acid is susceptible and unstable to heat, mechanical processes, oxidation, and light, which can destroy up to 90% of it [9, 10, 11]. Folic acid, with a molar mass of 441 Dalton/Da, and particle size of 0.001 – 0.01  $\mu\text{m}$  will pass through an MF membrane with a 0.15  $\mu\text{m}$  pore size [12, 13], which is more in permeate than retained in retentate except due to its occurrence of fouling [12]. MF membrane specifications include fluoropolymer-based pore sizes of 0.15 to 0.65  $\mu\text{m}$ , operating pressures of 1 to 10 bar (on a bench scale), temperatures of 0 to 60  $^{\circ}\text{C}$ , acidity range of 1 to 11, and the ability to filter out macromolecules greater than 500.000 g/mol or particles greater than 0.1 to 10  $\mu\text{m}$  [13]. Protein, fat, and polysaccharides are adhered on the surface of the MF membrane, while other substances with particle sizes less than 0.15  $\mu\text{m}$ , such as polyphenols, beta-carotene (0.1 - 10  $\mu\text{m}$ ), vitamins (folic acid, other vitamins), organic acids, amino acids, and minerals (0.001 - 0.1  $\mu\text{m}$ ), are allowed to pass freely through as permeate [13, 14, 15]. Composition and characteristics in retentate and permeate as well as membrane performance are influenced by MF process conditions (flow rate, operation pressure, temperature, time, and type of feed) [16], [17], [18], [19].

This study aims to ascertain the most suitable operating conditions in the CFMF process system (bench or module scale) by varying the type of feed, such as biomass of fermented nixtamal corn and separation process time at a fixed pump motor frequency (PMF) of 10 Hz or flow rate and TMP of 4 bar with retentate and permeate on chemical composition, in particular folic acid, identification of folic acid monomer, molecular weight, particle size and its distribution following the purification process for natural folic acid preparation.

## II. MATERIAL AND METHOD

### A. Materials and Equipment

The primary materials were dry horse dent yellow corn kernel obtained from a nearby market (South Tangerang), *Rhizopus oligosporus* strain C1 inoculum (Research Center for Chemistry, BRIN), *Rhizopus sp.* inoculum (PT. Aneka Fermentasi Indonesia), standard folic acid (Sigma Aldrich),  $\text{Ca}(\text{OH})_2$  (Merck), and microfiltration membrane with pore size 0.15  $\mu\text{m}$  (FSM-0.15-PP, Alfa Laval, Denmark). A series of laboratory-scale nixtamalization systems, a homogenizer (Ultra-Turrax, Ika Labortechnik, T50, Jane & Kunkel, Germany), microbiology system (laminar flow chamber and incubator), a plate and frame type crossflow membrane filtration module (Lab Unit M20, DSS, Denmark), and glassware were used in this study. The analysis instruments used in this study were UV-Vis Spectrophotometer (Model RF-550, Shimadzu, Japan), Liquid Chromatography coupled with Mass Spectrometry (LC-MS) (Mariner Biospectrometry) with LC (Hitachi L 6200) [20, 21, 22], and Particle Size Analyzer (PSA) using PSA Coulter SZ 100 (Horiba Nano Partica) [23], [24], [25].

### B. Experimental Design

The biomass feed of Fermented Nixtamal Corn (FNC) fermented by *Rhizopus oligosporus* strain C1 (FNC-A) or *Rhizopus sp.* (FNC-B) was passed through MF membrane

(0.15  $\mu\text{m}$ ) installed at plate and frame type crossflow membrane filtration module at room temperature, PMF of 19 Hz and TMP 4 bar for 0, 15, 30, 45, 60, 75, and 95 minutes. The biomass of fermented nixtamal corn, feed, and separation process results, including retentate and permeate, were analyzed for total solids (Gravimetric method), dissolved protein (Lowry method), total sugars (Phenol Sulphate method), and reducing sugars (Somogyi Nelson) [26], [27], [28], [29] and folic acid (UV-vis Spectrophotometer) [30, 31, 32]. LC-MS identified folic acid [20], [21], [22] and characterized by Particle Size Analyzer (PSA) with a PSA Coulter SZ 100 (Horiba Nano Partica) [23], [24], [25]. The research data was processed in duplicate according to description. The result of a triple average analysis is used for data processing.

### C. Procedure

The process of nixtamalization involved taking a certain amount of dry-shelled yellow corn known as horse dent corn. The corn was washed and then soaked in water using a ratio of 1 part corn to 4 parts water for a duration of 18 hours. A solution containing 20%  $\text{Ca}(\text{OH})_2$  (w/w, dissolved protein of corn) was added to the mixture. The mixture was heated to approximately 90 $^{\circ}\text{C}$  for 60 minutes and then cooled. Afterward, the cooked corn was rinsed to remove the cooking water and excess lime. The resulting material was ground and sifted through an 80-mesh sieve to obtain a powder known as nixtamal yellow corn powder (NYCP).

In addition, NYCP was inoculated with an *R. oligosporus* strain-C1 (0.2 %, w/w, nixtamalization corn) or *Rhizopus sp.* (0.2%, w/w, nixtamalization corn) inoculum, packed in ventilated plastic and stored at ambient temperature in fermentation rack for 36 hours to generate fermented nixtamal corn by *R. oligosporus* strain-C1 (FNC-A) and fermented nixtamal corn by *Rhizopus sp.* (FNC-B). The FNC product was then crushed in water at a 1:5 FNC/water ratio and filtered through a 100-mesh sieve to obtain filtrate as feed in the purification process by CFMF system equipped with MF membrane (0.15  $\mu\text{m}$ ). The FNC-A feed was placed in a 9 L holding tank with overflow and then pumped via 200 m of filter, heat exchanger, MF membrane with pore diameter of 0.15  $\mu\text{m}$  installed in membrane module, and retentate output. Feed or retentate was re-circulated to a holding tank.

In order to maintain a steady fluid temperature in the holding tank at room temperature (25  $^{\circ}\text{C}$ ), cooling water was flown to the heat exchanger during the circulation process at a temperature of 23 to 24  $^{\circ}\text{C}$ . Once the process was stable, the working pressure was set at 10 bar, and the pump motor frequency (PMF) was adjusted to 10 Hz (equivalent to a flow rate of roughly 3.5 L/min). Fluid that freely passes through each membrane area unit (permeated) via permeated housing was collected, its volume was measured, and its duration was recorded to calculate the permeate flow value. Permeate and retentate were collected at 0, 15, 30, 45, 60, 75, and 90 minutes. For FNC-B, the same process steps and process conditions were used. Investigation and sampling were conducted on permeate and retentate for 0, 15, 30, 45, 60, 75, and 90 minutes. Similar process procedures and process conditions were employed for FNC-B.

### III. RESULT AND DISCUSSION

#### A. Characteristics of materials

Nixtamal corn produced through the nixtamalization process using Ca (OH)<sub>2</sub> solution is granule with a softer texture and brighter yellow than dry corn kernel color and texture. The dry corn kernel and nixtamal corn both contain 95.83 and 179.12 µg/mL of folic acid, 0.97 and 1.88 mg/mL of dissolved protein, 91.49 and 46.73% of total solids, 2.25 and 11.41 mg/mL of total sugars, and 1.92 and 12.57 mg/mL of reducing sugars, respectively. This compositional difference illustrates the impact of the calcium hydroxide solution on the binding of each constituent, resulting in elevated levels of folic acid, dissolved protein, total sugars, and reducing sugar, while simultaneously reducing total solids.

Because of the gel-forming properties of corn starch arising from the amylose chain binding during the nixtamalization process, there is an enhancement in the content of folic acid and dissolved protein, leading to a reinforced structure of corn starch [33], [34], [35]. Similarly, folic acid and dissolved protein are suspected to bind strongly on the starch gel. The content of corn's amylose and amylopectin are approximately 28% and 72%, so it is easy for syneresis and retrogradation processes [34], [36], [37, 38] and increases total sugars. Increasing and reducing sugars enable autolysis at the tissue of corn granules by heat reaction, causing fractionation and increasing reduction property on the formation of monosaccharides.

Decreasing total solids is possibly caused by water absorption from tissue corn seed, so the system cannot retain the gelatinization process, which tends to dissolve components of corn and decreases total solids [37, 39]. Reducing sugars in feed is a factor in how often the corn polysaccharide (starch) is disrupted by bacteria naturally during the maize steeping process in water and heating by adding Ca (OH)<sub>2</sub> solution, both of which include heat reactions [33], [36], [37]. The Nelson-Somogyi method states that these processes can produce reducing sugars like glucose and fructose [26], [27], [28]. The monosaccharide formation and its reduction potential is facilitated by the non-reactive nature of the fractionation process achieved by adding Ca (OH)<sub>2</sub> solution. It is not easy to experience retrogradation and syneresis [33], [36], [37]. The solid fermentation process from nixtamal corn is mass filled by mold mycelia on the surface of nixtamal corn. The composition of FNC revealed that using *Rhizopus oligosporus* strain C1 (A) inoculum produced FNC-A with greater folic acid concentrations of 123.31 µg/mL, dissolved protein concentrations of 2.21 mg/mL, total sugars concentrations of 13.78 mg/mL, and reducing sugars concentrations of 11.12 mg/mL, compared to utilizing an inoculum of *Rhizopus* sp. (B) that produced FNC-B with folic acid concentrations of 117.5 µg/mL, dissolved protein concentrations of 2.11 mg/mL, total sugars of 6.52 mg/mL, and reducing sugar concentrations of 6.63 mg/mL. FNC-B yields higher total solids of 49.27% than FNC-A, with total solids of 47.98%. This discrepancy in total solids may be due to FNC-B having a denser bulk and thicker mycelia than FNC-A, resulting in higher total solids associated with various proteolytic and amylolytic activities.

The pulverizing process of fermented nixtamal corn to obtain feed showed a difference in composition. Feed of FNC-A and FNC-B was resulted in folic acid of 206.40 and 220.86 µg/mL, dissolved protein of 3.24 and 1.34 mg/mL, total solids of 4.67 and 3.56%, total sugars of 30.33 and 25.12 mg/mL, and reducing sugars of 18.68 and 13.68 mg/mL, respectively. This process alters the composition, especially dissolved protein, which tends to have a different composition than folic acid, reducing sugars, total sugars, and total solids. This process aims to facilitate the operation of a microfiltration membrane system so that material efficiency and recovery of valuable components from fermented nixtamal corn, particularly folic acid, can be achieved. The whole material composition is shown in Table 1. Meanwhile, Figure 1 shows subsequent compositions of dry horse dent yellow corn kernel, corn after the nixtamalization process, an inoculum of *Rhizopus oligosporus* strain C<sub>1</sub>, an inoculum of *Rhizopus* sp., fermented nixtamal corn by *Rhizopus oligosporus* strain C<sub>1</sub> (FNC-A), and fermented nixtamal corn by *Rhizopus* sp. (FNC-B).

TABLE I  
THE COMPOSITION OF NIXTAMALIZED CORN FERMENTED BY *RHIZOPUS OLIGOSPORUS* STRAIN C1 AND *RHIZOPUS* SP. AS NATURAL FOLIC ACID SOURCE

Type of materials	Composition				
	Folic Acid (µg/mL)	Dissolved Protein (mg/mL)	Total Solid (%)	Total sugar (mg/mL)	Reducing sugar (mg/mL)
Corn	95.83	0.97	91.49	2.25	1.92
Nixtamalized corn	179.12	1.88	46.73	11.41	12.57
FNC-A*	123.31	2.21	47.98	13.78	11.12
FNC-B*	117.50	2.11	49.27	6.52	6.63
Feed A**	206.40	3.24	4.67	30.33	18.68
Feed B**	220.86	1.34	3.56	25.12	13.68

Legend: \*) A, *Rhizopus oligosporus* C<sub>1</sub>, B, *Rhizopus* sp. in fermentation at ambient temperature for 36 hours; \*\*) filtration at a 1:5 corn-to-water ratio, sieved through 100 mesh prior to process.

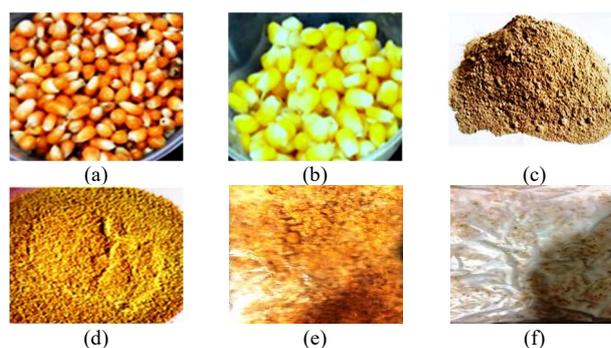


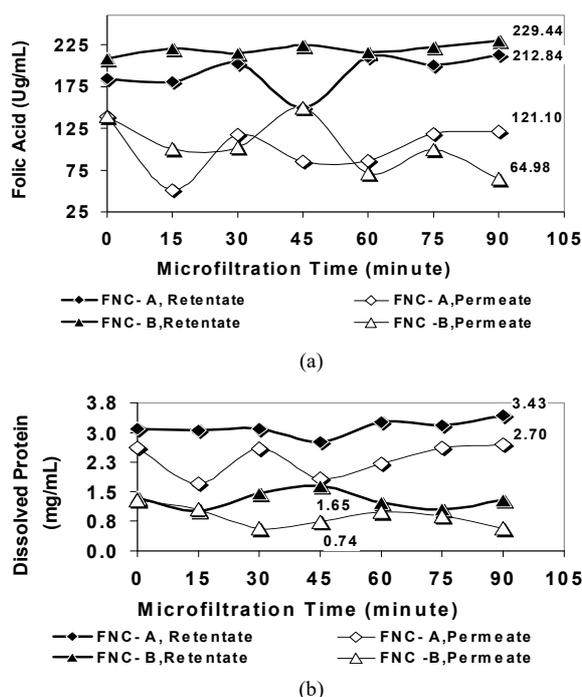
Fig. 1 (a) dried horse dent yellow corn kernel, (b) nixtamal corn, (c) *Rhizopus oligosporus* strain C<sub>1</sub> inoculum, (d) *Rhizopus* sp inoculum, (e) nixtamalized corn fermented by *Rhizopus oligosporus* strain C<sub>1</sub> (FNC-A), and (f) nixtamal corn fermented by *Rhizopus* sp. (FNC-B).

#### B. The influence of Microfiltration Process on Composition

1) *Folic acid and Dissolved Protein*: The separation of folic acid and dissolved protein takes place at varying rates, and with prolonged separation periods, an increased amount of folic acid and dissolved protein is retained in the retentate. However, they decrease in permeation. FNC-B yields a higher concentration of folic acid and a lower concentration of dissolved protein than FNC-A for all processing time. The separation process is going on successfully because folic acid

and dissolved protein are retained more in retentate than passing through permeate for all process times in both FNC-A and FNC-B, as shown in Figures 2a and 2b. Due to the presence of glutamic acid in its structure, folic acid is categorized as protein derivative [2], [3], [40], while dissolved protein is a mixture of amino acids or peptides and other compounds with a smaller particle size of 0.001 - 0.001  $\mu\text{m}$  [14], [15] compared to MF membrane pore size of 0.15  $\mu\text{m}$ . This smaller particle size should pass freely as permeate except when fouling forming cake occurs by polarization on the material particles on the membrane surface [12], [13], [14], [15], [16], [17], [18], [19].

The process conditions for MF are PMF 10 Hz with flow rate 3.5 L/min and TMP 4 bar, hence the only interaction between feed and separation time that is likely to have an impact on folic acid and dissolved protein concentrations in retentate and permeate. The optimum folic acid on FNC-B and FNC-A in retentate is obtained at 90 minutes of the separation process, obtaining folic acid of 212.84 and 229.44  $\mu\text{g/mL}$  and passing in permeate 121.10 and 64.98  $\mu\text{g/mL}$ . The CFMF process system, when operated under these optimum conditions, elevated the content of folic acid in the retentate of FNC-A (33.77%) and FNC-B (95.27%) as compared to the amounts of folic acid in FNC-A (123.31  $\mu\text{g/mL}$ ) and FNC-B (117.50  $\mu\text{g/mL}$ ) biomass.



Legend : \*) A, by *Rhizopus oligosporus* strain C<sub>1</sub> and B, by *Rhizopus* sp.

Fig. 2 The relationship of material types and microfiltration time on (a) folic acid and (b) dissolved protein through MF membrane (0.15  $\mu\text{m}$ ) with PMF 10 Hz and TMP 4 bar at ambient temperature.

On dissolved protein in retentate, optimization of FNC-A and FNC-B is reached for 90 and 45 minutes of separation time, respectively. The difference in this optimization is possibly related to the difference in the initial concentration of dissolved protein and the interaction between the operation conditions of the membrane (flow rate, TMP). In fixed operation conditions (PMF 10 H/flow rate  $\sim$ 3.5 L/min., TMP 4 bar), the difference in dissolved protein concentration of

feed gives the possible direct effect, in which feed with higher dissolved protein concentration (FNC-A, 3.24 mg/mL) causes more reactive system so that it does not give the opportunity of dissolved protein to pass as permeate. In contrast, on the feed of FNC-B with lower dissolved protein concentration (1.34 mg/mL), dissolved protein is trapped faster in forming cake (45 min) followed only by a part of dissolved protein retained on retentate due to lower initial concentration. However, the longer the separation process duration, the higher the level of permeation passing in. Both materials are enabled to interact between PMF (10 Hz or equivalent to flow rate of 3.5 L/min.) and TMP (4 bar). The dissolved protein content in FNC-A and FNC-B is optimized after 90 and 45 minutes of separation, respectively, generating retentate with dissolved protein concentrations of 3.43 and 1.65 mg/mL and passing in permeate with concentrations of 2.70 and 0.74 mg/mL, respectively. The CFMF process system can increase dissolved protein concentration in FNC-A retentate (55.20%) and decrease dissolved protein concentration in FNC-B retentate (21.80%) in this optimal operating condition, compared to dissolved protein concentration in FNC-A biomass (2.21 mg/mL) and biomass of FNC-B (2.11 mg/mL).

2) *Total sugars and Reducing sugar*: According to the Phenol Sulphate method, total sugars are the entire amount of carbohydrates found in FNC. Meanwhile, reducing sugars is a sugar molecule that can reduce because hydroxyl (-OH) groups have reactive properties according to the Nelson-Somogyi method [26, 27, 28, 29]. Reducing sugars indicates the activity of amylolytic fungi in converting corn polysaccharides, including glucose or fructose [41,42]. Total sugars and reducing sugars are part of the concentrate composition as natural folic acid preparation from FNC. Process time becoming increasingly long increases total sugars in retentate for both types of feed, in which the reaction rate fluctuates. However, the separation process continues because more total sugars are kept on the top surface of the membrane than pass as permeate. Total sugars in retentate of FNC-A fluctuate and have the highest concentration for 75 min, followed by dropping. In contrast, total sugars in the retentate of FNC-B increase to 90 min. of separation process time. The passing rate of total sugars in permeate shows a different result, in which total sugars in permeate of FNC-A increases.

Meanwhile, total sugars in the permeated of FNC-B fluctuate and decrease more and more to 90 min. of separation process time, as indicated in Figure 3a. The difference in the concentration of this total sugar is not only caused by the bigger size of sugar particles (such polysaccharides) ranging from 0.0008 - 0.001  $\mu\text{m}$  [13, 14] so that occurs *fouling* [19, 43, 44] but also caused by the initial concentration of material of FNC-A both biomass (13.78 mg/mL) and higher feed (30.33 mg/mL) compared with the initial concentration of material of FNC-B both biomass (6.52 mg/mL) and feed (25.12 mg/mL). In this fixed operation condition (PMF 10 H/flow rate  $\sim$ 3.5 L/min., TMP 4 bar), the difference in concentration of components in material tends to affect the separation rate. Optimization of total sugars in FNC-A and FNC-B is achieved for 75 min and 90 min. of separation process time generate retentate with concentrations of total sugars of 36.85 and 34.34 mg/mL, respectively, and passing in permeate is 22.85 and 4.44 mg/mL, respectively. Under this

optimum condition, the CFMF process system increases the total sugars in retentate of FNC-A by 167.42%, or 1.7-folds, and FNC-B by 426.69%, or 4.27-folds, in comparison to the total sugars in biomass of FNC-A (13.78 mg/mL), and FNC-B (6.52 mg/mL).

Different tendencies look at the separation process of reducing sugars. The separation rate fluctuates unsuccessfully for FNC-A because it passes more reducing sugars in permeate than retentate. As demonstrated in Figure 3a, more reducing sugars are retained in retentate than flow through permeate, therefore the separation rate of reducing sugars for FNC-B continues to proceed satisfactorily. It is reducing sugars as glucose or fructose range  $0.0008 - 0.001 \mu\text{m}$  [13, 14], so they will pass more in permeate. Reducing sugars in the feed of FNC-A to 18.68 mg/mL so that it occurs to foul rapidly until 30 min. They were followed by declining to final separation process time. Meanwhile, reducing sugars in the feed of FNC-B was 13.68 mg/mL, so fouling occurred during the final separation process, as shown in Figure 3b. In process condition of PMF 10 Hz (equivalent to flow rate of 3.5 L/min) and TMP 4 bar, the difference in reducing sugars concentration in feed is a factor affecting on separation process time increasing. After 30 and 90 minutes of separation, respectively, the reducing sugars in FNC-A and FNC-B are optimized, yielding reducing sugar concentrations of 16.53 and 17.96 mg/mL in the retentate and 18.08 and 2.76 mg/mL in the permeate. When compared to the reducing sugars in the respective compounds' biomasses (11.12 mg/mL and 6.63 mg/mL), the CFMF process method increased the reducing sugars in the retentate of FNC-A (48.65%) and FNC-B (170.89% or 1.71-folds).

Additionally, the CFMF system successfully separated FNC-A and FNC-B since more total solids were held in retentate than were passed in permeate, as shown in Figure 3c. According to the Gravimetric method, total solids are total collected materials that include the part of total solids retained and total dissolved solids. The total solids in the retentate increase as the process time increases. However, it decreased the total solids in permeate at both types of FNC until the final process time (90 minutes). FNC-A shows a higher concentration of total solids than FNC-B for all separation process time. This matter is brought on by the fact that total solids in the feed of FCN-A are more concentrated (4.67%) than in the feed of FCN-B (3.56%), and as a result, materials on the membrane surface are more polarized. Total solids in FNC-A and FNC-B are optimized for 90 minutes of separation with concentrations of 5.32 and 4.12% in retentate and 2.49 and 0.84% in permeate, respectively. The CFMF process system reduces total solids concentrations in retentate of FNC-A (88.91%) and FNC-B (1.64%) to total solids concentrations in biomass of FNC-A (47.98%) and FNC-B (49.27%) at this optimum operation state. This decrease in total solids is caused by diluting biomass (1 part) with water (5 parts) in feed preparation. Increasing total solids takes place by components polarization on the top surface of the membrane in generally circa >30% from the whole component caused by interaction amongst feed, TMP as driving force, and flow rate in the CFMF process system [15, 16, 17].

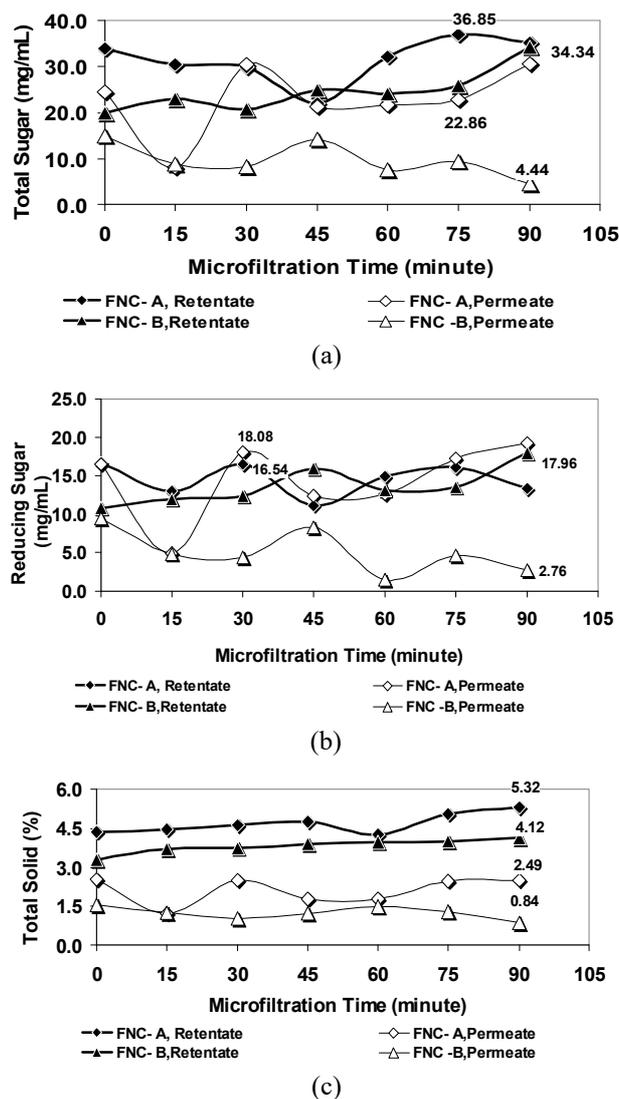


Fig. 3 The relationship of material types and microfiltration time on (a) total sugars, (b) reducing sugar, and (c) total solids through MF membrane at ambient temperature, PMF 10 Hz and TMP 4 bar for 90 min.

### C. Optimum Operating Process of Microfiltration

Based on achieving the highest folic acid concentration in the retentate, the optimal process conditions for separating components in biomass FNC-A and FNC-B are determined to be a fixed operation condition with a process time of 90 minutes (PMF 10 Hz/flow rate 3.5 L/min. and TMP 4 bar). This process condition produced retentate of FNC-A and FNC-B with folic acid concentrations of 212.84 and 229.44  $\mu\text{g/mL}$ , dissolved protein concentrations of 3.43 and 1.28 mg/mL, total sugars of 35.21 and 34.34 mg/mL, reducing sugars concentrations of 13.39 and 17.96 mg/mL, total solids concentrations of 5.32 and 4.12%, whereas FNC-A and FNC-B permeates have folic acid concentrations of 121.10 and 64.97 mg/mL, dissolved protein concentrations of 2.70 and 0.57 mg/mL, total sugars of 30.65 and 4.44 mg/mL, reducing sugars concentrations of 19.27 and 2.76 mg/mL, and total solids concentrations of 2.49 and 0.84%, respectively. CFMF process system can increase valuable components in the retentate of FNC-A and retentate of FNC-B, including folic acid of 72.61% and 95.26%, reducing sugar of 20.41% and 170.93% (1.7-folds), total sugars of 155.51% (1.55-folds) and

426.76% (4.27-folds), and dissolved protein of 55.33% and 39.20%, and drop total solids of 88.91% and 91.64%, respectively, compared to the concentrations of each component in FNC-A and FNC-B biomass.

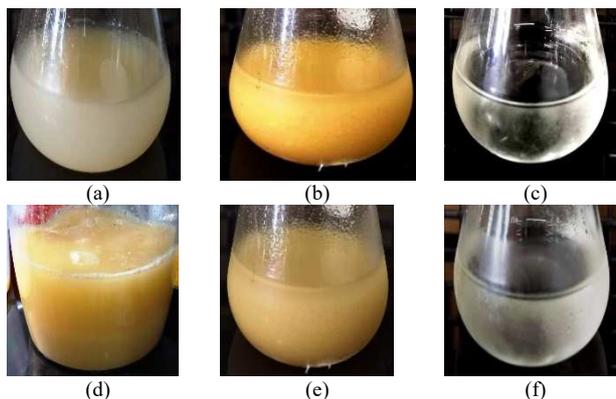


Fig. 4 (a) feed FNC-A, (b) retentate FNC-A, (c) permeate FNC-A, (d) feed FNC-B, (e) retentate FNC-B and (f) permeate FNC-B through MF membrane at ambient temperature, PMF 10 Hz and TMP 4 bar for 90 min.

#### D. Characterization of folic acid monomer in fermented nixtamal corn

The optimum condition, in which yields FNC-A and FNC-B after a 90-minute separation process, is used to identify the folic acid monomer. By using LC-MS technique, it was found that folic acid has a molecular weight (MW) of 441 Dalton (Da) [11]; a compound's MW difference reveals its potential state as  $M^+$ ,  $M^+ Na^+$ ,  $2M^{++}$ , or  $2M^+$ ,  $Na^+$ . The LC-MS analysis revealed that folic acid exhibits a molar mass of 441 Dalton (Da.) [11], and a compound exhibits discrepancies in molar mass, which may manifest as  $M^+$ ,  $M^+ Na^+$ ,  $2M^{++}$ , or  $2M^+$ ,  $Na^+$  [12]. In this analysis result, this folic acid monomer is used as  $M^+$  [20, 21, 22]. The LC-MS operating parameters include a volume injection of 5 L and a flow rate of 0.2 mL/min utilizing a column C-8 (15 mm x 2 mm) and an eluent mixture of methanol and water in an 80:20 ratio. Figure 5 depicts the overall chromatogram and mass spectra.

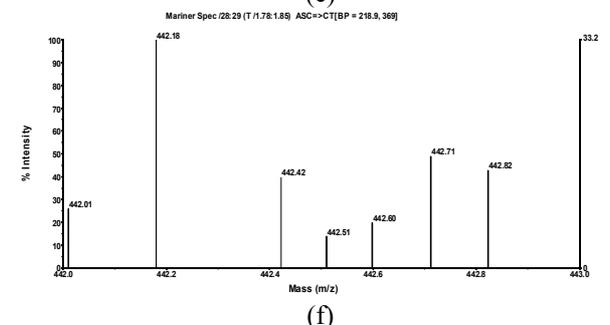
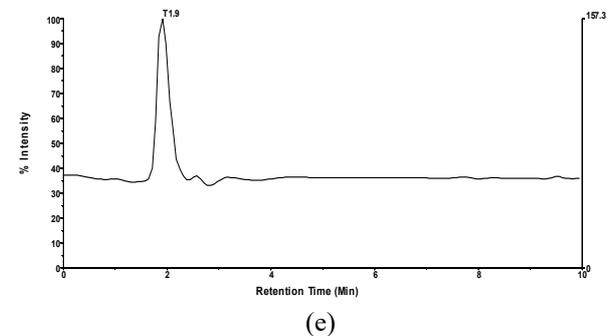
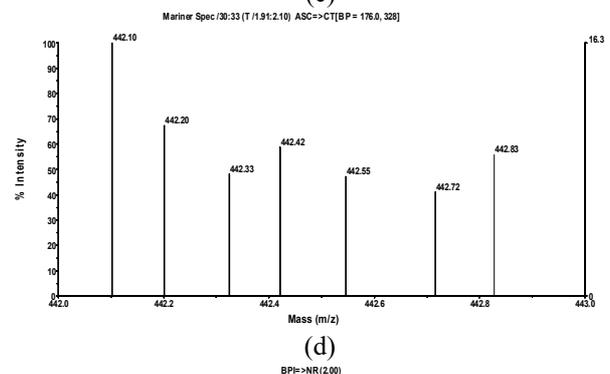
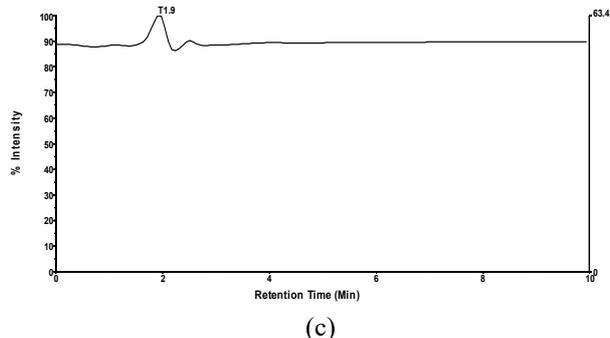
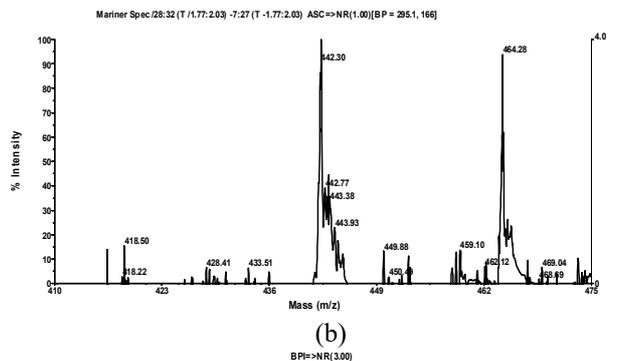
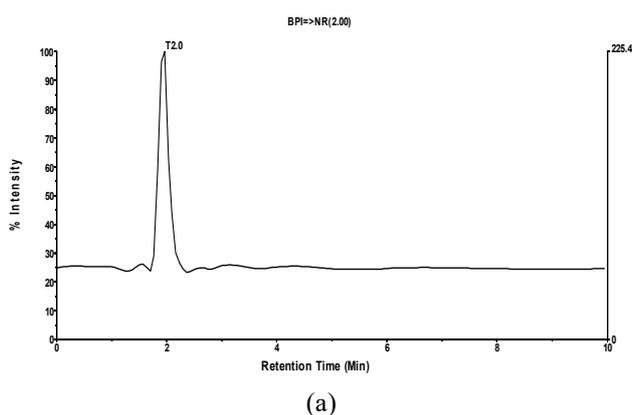


Fig. 5 Chromatogram of standard folic acid (a), mass spectra from T 2.0, (c) chromatogram of FNC-A, (d) mass spectra of FNC-A, (e) chromatogram of FNC-B, and (f) mass spectra of FNC-B through MF membrane at room temperature, PMF 10 Hz and TMP 4 bar for 90 min

The standard folic acid sample exhibited a solitary peak (T2.0) that appeared between 0 and 10 minutes of retention time, demonstrating a relative intensity of 100%. The mass spectra originating from T2.0, as depicted in Figures 5a and 5b, exhibit a prominent presence of the MW monomer at 442.30 and 442.77 Da., showing relative intensities of 100% and 40%, respectively. The chromatograms originating from the retentate of both feeds, shown in Figures 5c and 5e, exhibit a single peak (T 1.9) observed during the retention time of 0 - 10 min., with a relative intensity of 100%. The mass spectra of FNC-A at T 1.9, presented in Figures 5d and 5f, confirm the prevalence of folic acid monomers with molecular weights of 442.10 Da and 442.18 Da, displaying relative intensities of 100% within the m/z range of 442 to 443.

### E. Effect of Process Condition on Particle Size and Particle Size Distribution

FNC-A and FNC-B feed suspensions are produced by filtering the pulverized material through a 100 mesh sieve, using a ratio of 1 part FNC-A or FNC-B to 4 parts water. Meanwhile, the retentate of FNC-A and FNC-B are suspended from treatment at optimum process conditions (PMF of 10 Hz/flow rate ~3.5 L/min., TMP 4 bar, 90 min.), which is sufficient thick suspension with yellowish, respectively. Feed and concentrate from treatment for 90 minutes of optimum time yield particle size, as shown in Table 2.

TABLE II  
AVERAGE PARTICLE SIZE FROM FEED AND RETENTATE OF  
FNC-A AND FNC-B

Type of material	Z-Average ( $\mu\text{m}$ )*	Z-Average ( $\mu\text{m}$ )*	Z-Average ( $\mu\text{m}$ )*
Feed FNC-A	58.64	Feed FNC-B	36.01
Retentate FNC-A	38.31	Retentate FNC-B	37.97

Legend : \*Diameter of particles.

The feed of FNC-A resulted in a larger particle size (58.64  $\mu\text{m}$ ) than that of retentate (38.31  $\mu\text{m}$ ), while the feed of FNC-B yielded a smaller particle size (36.01  $\mu\text{m}$ ) than that of retentate (37.97  $\mu\text{m}$ ). The change in particle size could be caused by feed suspension passing through a 100-mesh sieve so that larger particle size compared with concentrate as a result of microfiltration membrane process, in which CFMF process system passes also a part suspension as permeate. Particle size is affected by the composition of each material accumulated as total solids. Total solids in the feed of FNC-A (18.68%) yield retentate with total solids of 5.32%, whereas total solid in the feed of FNC-B (13.68%) generates retentate of 4.12% so that the particle size of FNC-A tends larger than that particle size of feed and retentate of FNC-A.

On the distribution of particles, feed and concentrate of FNC-A yield distribution of particles with particle diameter ( $\emptyset$ ) size of 0.011 – 88.58  $\mu\text{m}$  (<100  $\mu\text{m}$ ) at a frequency (q) of 49 – 81%, particle diameter ( $\emptyset$ ) size of 101.46 – 890  $\mu\text{m}$  (<1000  $\mu\text{m}$ ) at a frequency (q) of 56 – 100%; and particle diameter ( $\emptyset$ ) size of 1019 – 5000  $\mu\text{m}$  (<10000  $\mu\text{m}$ ) at a frequency (q) of 51 – 100% or in other words, feed of FNC-A and retentate of FNC-A show distribution of particle at 10, 50 and 90% from all particles subsequently of 7.37  $\mu\text{m}$ , 16.48  $\mu\text{m}$ , 188.47  $\mu\text{m}$  and 10.31  $\mu\text{m}$ , 26.32  $\mu\text{m}$ , and 81.55  $\mu\text{m}$ , as demonstrated in Figure 5a and 5b. Feed and retentate of FNC-B resulted in particle distribution with particle diameter ( $\emptyset$ )

sizes of 0.011 – 88.58 (<100  $\mu\text{m}$ ) at a frequency (q) between 11 and 49%, particle diameter ( $\emptyset$ ) sizes of 101.46 – 890  $\mu\text{m}$  (<1000  $\mu\text{m}$ ) at a frequency (q) between 56 and 100%, and particle diameter ( $\emptyset$ ) size of 1019 – 5000  $\mu\text{m}$  (<10000  $\mu\text{m}$ .) at a frequency (q) between 51 and 100% or in other words, feed of FNC-B and retentate of FNC-B shows the distribution of particle at 10, 50 and 90% from all particles subsequently of 7.25, 14, 17 and 127.00  $\mu\text{m}$ , and 10.37, 28.04 and 76.09  $\mu\text{m}$ , as shown in Figure 5c and 5d. This indicates that the MF membrane process affects particle size and distribution. The purification process at PMF 10 Hz/flow rate 3.5 L/min and TMP 4 bar for 90 min of separation process time at room temperature resulted in the retentate of FNC-B having a larger range of particle distribution when compared with the retentate of FNC-B.

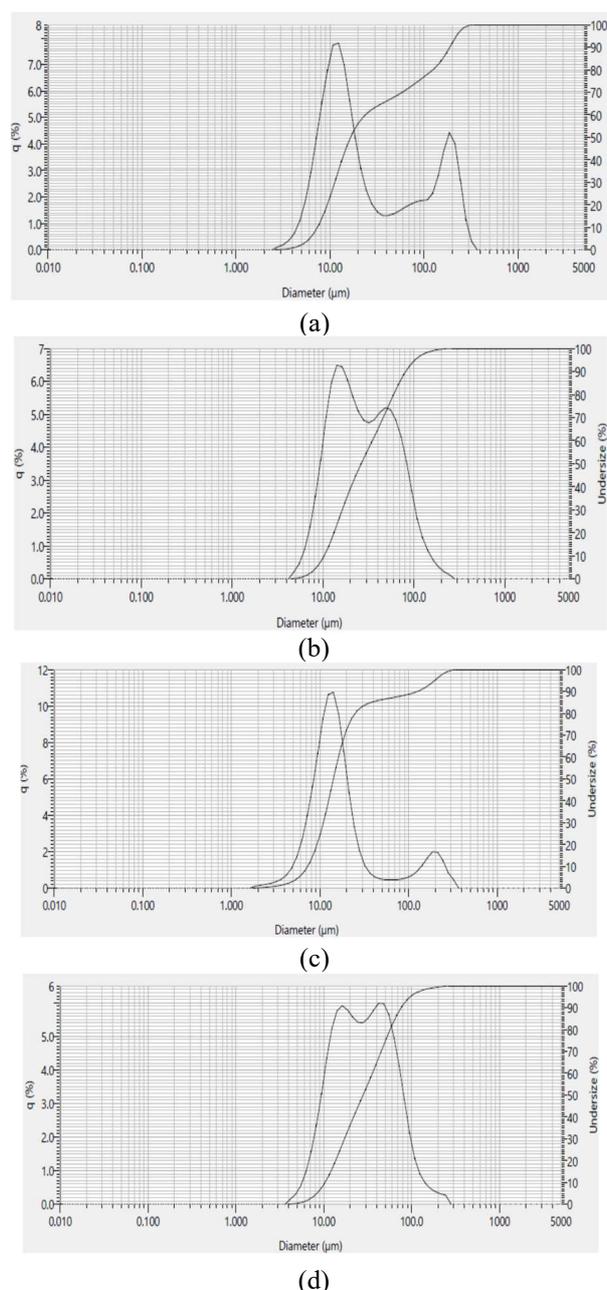


Fig. 6 Distribution particles of (a) feed of FNC-A, (b) retentate of FNC-A, (c) feed of FNC-B, and (d) retentate of FNC-B through MF membrane at PMF 10 Hz and TMP 4 bar for 90 min.

#### IV. CONCLUSION

The biomass FNC-A and FNC-B separation process utilizing the CFMF system for different process times and types of feed at fixed conditions (PMF 10 Hz/flow rate ~3.5 L/min., TMP 4 bar) was successfully able to separate folic acid, dissolved protein, and total solids. However, it was unsuccessful for total sugars and reducing sugars. Process time becoming increasingly long increases all the components in the retentate. However, the fluctuating part content permeates for both biomasses is due to the extended process duration. The 90-minute separation process yielded the optimal conditions for separating FNC-A and FNC-B in the retentate, as determined by the highest folic acid performance. These circumstances produced retentates of FNC-A and FNC-B with folic acid contents of 212.84 and 229.44 µg/mL, dissolved protein contents of 3.43 and 1.28 mg/mL, total sugars contents of 35.21 and 34.34 mg/mL, reducing sugar contents of 13.39 and 17.96 mg/mL, and total solids contents of 5.32 and 4.12%.

While the folic acid concentrations in FNC-A and FNC-B permeates were 121.10 and 64.97 µg/mL, respectively, as well as 2.70 and 0.57 mg/mL for dissolved protein, 30.65 and 4.44 mg/mL for total sugars, 19.27 and 2.76 mg/mL for reducing sugar, and 2.49 and 0.84% for total solids. Under these optimal conditions, the CFMF system effectively enhanced the folic acid content in retentate FNC-A and retentate FNC-B by 72.61% and 95.26%, reducing sugars by 20.41% and 170.93% (1.7-folds), total gula of 155.51% (1.55-folds) and 426.76% (4.27-folds), dissolved protein of 55.33 and 39.20%, however, it decrease total solids by 88.91% and 91.64% compared with the concentration of components in biomass of FNC-A and FNC-B. In this optimal condition, the retentates of FNC-A and FNC-B were primarily composed of folic acid monomers with molecular weights of 442.10 Da and 442.18 Da, respectively, exhibiting a relative intensity of 100%. Moreover, the average particle sizes of FNC-A and FNC-B were measured to be 38.31 µm and 37.97 µm, respectively, and distribution of particle size at 10, 50 and 90% from all particles were 10.31, 26.32, 81.55 µm and 10.37, 28.04 and 76.09 µm, respectively.

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