Difference in Characteristic of Concentrate Powder of Corn (Zea mays var. indentata) Fermented by Bifidobacterium brevis as Natural Folic Acid Fortificant

Aspiyanto\textsuperscript{1,*}, A Susilowati\textsuperscript{1}, P D Lotulung\textsuperscript{1} and Y Maryati\textsuperscript{1}

\textsuperscript{1}Research Centre for Chemistry, Indonesian Institute of Sciences, I452 Building, Kawasan PUSPIPTEK, Serpong - 15314, South Tangerang, Banten, Indonesia

*Corresponding author: aspiyanto_2010@yahoo.com

Abstract. Both white and yellow corns (Zea mays var. indentata) fermented by Bifidobacterium brevis has potential use as fortificant of natural folic acid. This experiment activity aims to find out difference in characteristic between concentrate powder of white corn and yellow corn fermented by Bifidobacterium brevis subsequently through processes of nixtamalization with lime solution Ca(OH)\textsubscript{2}, fermentation by B. brevis culture, microfiltration (MF) (pore size 0.15 µm) by means of standard dead-end stirred filtration cell (DESFC) at room temperature, rotation speed 300 rpm and 300 rpm, and trans membrane pressure (TMP) 20 and 40 psia for 30 minutes, respectively, and drying at 50 °C for 36 hours. The result of experiment showed that a series of processes mentioned above gave difference in characteristic from two types of biomass concentrate powder with compositions of folic acid 565.35 µg/mL and 614.92 µg/mL, total solids 96.60 mg/mL and 96.76 mg/mL, dissolved protein 0.19 mg/mL and 0.30 mg/mL, reducing sugars 1.718 mg/mL and 59.300 mg/mL, total sugars 2.54 mg/mL and 59.00 mg/mL, and total acid 0.29 % and 0.41%, respectively. In this condition, biomass concentrate powder of A and B displayed domination of folic acid monomer with molecular weight (MW) 442.07 Dalton (Da.) and 442,72 Da. at relative intensity 75 % and 100 %, particles size 1115.1 nm and 461.0 nm, and Index Particles 0.827 and 0.653, respectively.

1. Introduction

Nixtamalization horse dent corn (Zea mays var. indentata) fermented by Bifidobacterium brevis as source of natural folic acid is novelty fermented food having potential prospect as fortificant on fermentation products for various types of food and supplement in form of powder, paste and suspension. Corn (Zea mays L.) is one of the best cereal grain crops or staple food crop for human consumption as polysaccharides source for energy and body builder, besides its functional properties as source of adequate high protein (± 11%) [1, 2], potential source of folic acid, and potential source of other bioactive compounds (β-carotene/provitamin A, etc.). Folic acid is an important factor in varying body functions started from nucleotides synthesis to remethylation homocysteine, particularly important period in cells breeding and growth. Children and adult need folic acid to produce red blood cells and prevent anemia [3]. Folic acid has main role as an essential co-enzyme factors in synthesis of
methionine, purine and pyrimidine. Only one function from folate-dependent co-enzymes in body is as a mediator in transferring one-carbon (C1) units functioning as C1-donors and acceptors in various most important enzymatic reactions on metabolism of nucleic acid (DNA) and amino acids. As source of folic acid, nixtamalized corn is potential substrates needed for growing Lactic Acid Bacteria (LAB). Mechanism on formation of folic acid in fermentation by *Bifidobacterium brevis* is possibility caused by its occurrence of biosynthesis folates de novo, as performed [4] by activity of other LAB. *Bifidobacterium brevis* generates also other metabolites, such as organic acids, particularly lactic acid by enzyme activity of β-D-Galactosidase followed by a less number of CO2, taste and specific aroma of yoghurt. Carbohydrate is source of carbon for growing *Bifidobacterium brevis* followed by getting sucrose and (skimmed) milk [5]. Modification treatment on nixtamalization process using Ca(OH)2 increases folic acid as a consequence of increase and adsorption of Ca(OH)2 [6]. A microfiltration (MF)-based separation process, which is applied to separate or fractionate or purify or concentrate target and desired components in biomass through MF membrane (0.15 µm pore size) fitted in dead-end stirred filtration cell (DESFC) under room temperature, stirrer rotation speed 300 rpm, and transmembrane pressure (TMP) 20 psia and 40 psia for 30 minutes generates two liquids (retentate or concentrate and permeate) with difference in their characteristic and composition [7]. MF membrane technique is selected because of advantages in order to anticipate loss of folic acid which is sensitive on heat, light, high acidity and mechanic treatment, no phase changing, high flexibility, and smaller space and easier in operation. In many cases, the low energy consumption, reduction in number of processing steps, greater separation efficiency and improved final product quality are the main attractions of this process [7, 8]. MF membrane technique allow retained folic acid more much on the membrane surface compared to permeate, although particle size of folic acid is smaller than that pore size of MF membrane used (0.01 µm < 0.15 µm) [9, 10]. The occurrence of fouling is possibility caused by operation condition factor, such as trans membrane pressure (TMP) and process time, type of membrane, and characteristic of biomass [11]. To get biomass concentrate powder having potency as fortificant, it is performed drying at 50 °C for 24 – 36 hours by means of cabinet dryer. This process is enabled to be affect on composition, particles distribution, and particle size. Smaller particles size with larger distribution enables to get better surface tension on formulation so that it facilitates its application process and adsorption folic acid by body.

This experiment activity aims to find out difference in characteristic (composition, particle distribution, folic acid monomer) of biomass concentrate powder from white corn and yellow corn fermented by *Bifidobacterium brevis* culture with different inoculum specification yielded through result of microfiltration as fortificant natural folic acid source.

2. Materials and Methods

2.1. Materials and Equipments

Main materials used in this experimental work were dry yellow corn and dry white corn (*Zea mays* var. *indentata*) purchased from a local market, culture of *Bifidobacterium brevis* (FNCC-UGM), Ca(OH)2 (E.Merck), standard folic acid (E.Merck), skimmed milk (Benato, USA), sucrose (local), DeMan-Rogosa-Sharpe (MRS A) medium, DeMann Rogosa Sharpe Broth (MRS B) medium, distilled water, technically nitrogen gas, and Commercial Fluoro polymer Microfiltration (MF) membrane (pore size of 0.15 µm, FSM-0.15-PP, Alfa laval, Denmark). Meanwhile, chemicals used in this process and analysis were standard folic acid (Aldrich), 3-aminophenol (Aldrich), hydrochloric acid (E.Merck), sodium nitrite (E.Merck), sulphamic acid (E.Merck), and methanol (E.Merck). All the chemicals used in this process and analysis were reagent grade, procured locally, and used without further purification.

Main equipments utilized in this experimental activity were Balance (Fujitsu, Japan), series of nixtamalization process system in laboratory scale, such as pan, blender (National, local), homogenizer (Ultra-Turrax, Ika Labortechnik, T50, Jane & Kunkel, Germany), cylindrical tank for technical nitrogen (Local), pressure gauge and regulator of technical nitrogen (Fisher Scientific Company, England), Dead-End Stirred Microfiltration Cell (SMFC) with capacity of 200 mL (MILLIPORE, Amicon Model 8200,
U. S. A.), magnetic stirrer (HI 303 N, HANNA Instrument, Japan), stop watch (Hanhart Profil 2, Germany), cabinet dryer (local), equipments of microbiology process, such as autoclave (CHENG YI, LS – 50 L, China), laminar air flow (local), incubator (local), and sieve of 80 mesh (Retsch, Germany). Main instruments for analysis were UV-vis Spectrophotometer (Model RF-550, Shimadzu, Japan), and Liquid Chromatography coupled with Mass Spectrometer (LC-MS) (Mariner Biospectrometry) equipped with LC (Hitachi L 6200) and Particle Size Analyzer (PSA) using PSA Coulter SZ 100 (Horiba Nano Partica).

2.2. Experimental Design
The experiment activity was conducted fermentation of nixtamalized white corn and nixtamalized yellow corn by inoculum of Bifidobacterium brevis at concentration 40% (v/w dissolved protein) and 40 °C for 24 hours so that it is get biomass A (nixtamalized white corn) and biomass B (nixtamalized yellow corn). Biomasses was further microfiltered on membrane (pore size of 0.15 µm) fitted in standard dead-end stirred filtration cell (DESFC) at room temperature, stirrer rotation speed 300 rpm and 300 rpm, and TMP 20 psia and 40 psia for 30 minutes, respectively. Since feed stream is forced by nitrogen gas through the membrane, it separates into two streams that differ in their composition, known as retentate (concentrate) and permeate. Retentate (concentrate) was then dried by using cabinet dryer at 50 °C for 36 hours so that it is yielded biomass concentrate powder A and B. Analysis was carried out on dissolved protein (Lowry method) [12], total solids (Gravimetric method), reducing sugars (Somogyi-Nelson), total acids (titratable acids method) [13] and folic acid (spectrophotometric UV-vis) [14] Ruengsitagoon and Hatthanat, 2012). Identification of folic acid (FA) monomer was conducted by means of LC-MS [15] and particle distribution was done by PSA Coulter SZ 100 [16] Backman, 2018). Process and analysis were conducted in duplicate. Data processed in this description were based on the result of average analysis.

3. Procedure
3.1. Nixtamalization of corn
A number of yellow corn and white corn from type of horse dent with variety of Zea mays var. indentata were subsequently cleaned, separated from dirt, washed, steeped in water at the corn-to-water ratio of 1 part : 4 parts for 18 hours by adding Ca(OH)\(_2\) at concentration 30% (yellow corn) and 20% (white corn), blanched 30 minutes (white corn) and 60 minutes (yellow corn), cooled to room temperature, and pulverized by blender at full speed in order to result nixtamalized corn pulp [6].

3.2. Preparation of Bifidobacterium brevis stock cultures, corn folic inoculum and fermentation of nixtamalized corn
Preparation of B. brevis stock cultures was performed by weighing MRS (Man-Rogosa-Sharpe) Broth 15.6 gram, adding aquadest 300 mL and autoclaving at 121 °C for 15 minutes, cooling and inoculating with starter of Bifidobacterium brevis 1% (w/v MRS B), incubating at 37 °C for 24 – 36 hours until the growth of microbes on media was seemed. Preparation of inoculum of white corn (A) was conducted by mixing one (1) part of nixtamalized white corn pulp and 2 parts of water, autoclaving at 121 °C for 15 minutes, cooling at room temperature, inoculating with Bifidobacterium brevis 40% (v/w in dissolved protein of nixtamalized corn) and fermenting at 40 °C for 24 hours. Preparation of yellow corn (B) inoculum was carried out by mixing one (1) part of nixtamalized yellow corn pulp and 2 parts of water, adding 10% (w/w) sucrose and 10% (w/w) skimmed milk, autoclaving at 121 °C for 15 minutes, cooling at room temperature, inoculating with Bifidobacterium brevis 40% (v/w in dissolved protein of nixtamalized corn), and fermenting at 40 °C for 24 hours (Susilowati et al., 2016). Fermentation of nixtamalized white corn was done by autoclaving nixtamalized white corn pulp (400 mL) at 121 °C for 15 minutes, cooling at room temperature, inoculating with 40% (v/w dissolved protein) inoculum A, and incubating at 40 °C for 24 hours so that it is yielded biomass A. Other treatment, a number of nixtamalized white corn pulp (400 mL) was added with 10% (w/w) sucrose and (10% w/w) skimmed...
milk, autoclaved at 121 °C for 15 minutes, cooled at room temperature, inoculated with 40% (v/w dissolved protein) inoculum of corn B, and incubated at 40 °C for 24 hours until it is generated biomass B.

3.3. Processes of purifying, drying and size reduction
Purifying biomass A and biomass B was performed by sieving through a 200 mesh to get filtrate introduced as feed, microfiltered through membrane a 0.15 µm fitted in standard DESFC under room temperature, stirrer rotation speed 300 rpm and 300 rpm, and TMP 20 and 40 psia for 30 minutes, respectively until it is resulted retentate (concentrate) and permeate (extract) [17]. Retentate (concentrate) was further dried in cabinet dryer at 50 °C for 36 hours, size reduction, and sieved via a 80 mesh so that it is get biomass concentrate powder A and biomass concentrate powder B.

3.4. Analysis of folic acid
Folic acid was analyzed by means of spectrophotometry according to diazotization reaction on p-aminobenzoylglutamic acid yielded after reduction reaction of folic acid and 3-aminophenol to form yellow-orange complex. One (1) mL of standard folic acid sample was added subsequently with 1 mL of Hydrochloric acid (4 M), 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 then measured through UV-Vis Spectrophotometer at wave length 460 nm [14].

3.5. Identification of folic acid by means of LC-MS
Suitable aliquots of samples contains biomass concentrate powder A and biomass concentrate powder B, and standard folic acid (FA). Oligomer was analyzed by a LC-MS using Minerv Biospectrometry. LC system was integrated with Q-TOP MS through Electrospray Ionization (ESI) system, in which scan mode was performed in a range of m/z 100 – 1200 at 140 °C. LC (Hitachi L 6200) was carried out on a C18 column from Supelco (15 mm x 2 mm) and particles size of 5 µm. Solvent types were a mixture of 80 parts of methanol and 20 parts of water with flow rate 0.1 mL/minute and injection volume of 5 uL [15].

4. Results and Discussions

4.1 Characteristics of Materials
Both biomass concentrate A and biomass concentrate B are the purification result of fermented nixtamalized white corn and fermented nixtamalized yellow corn by *Bifidobacterium brevis* inoculum with different inoculum. These result of purifications stated as biomass A and biomass B, respectively are generated by 40 % inoculum concentration without and with addition to 10 % skimmed milk and 10 % sucrose at 40 °C for 24 hours of fermentation and gives different component concentration. Compositions of biomass A and biomass B have folic acid 48.11 and 49.22 µg/mL, total solids 7.51 and 9.90%, dissolved protein 0.11 and 0.09 mg/mL, total sugars 10 and 14.2 mg/mL, reducing sugars 7.4 and 9.1 mg/mL, and total acids 0.05 and 0.195 mg/mL, respectively [5]. These compositions are influenced by process condition of fermentation (temperature and time) and initial composition from each component, and interaction between concentration and type of inoculum. Fermentation of nixtamalized yellow corn by inoculum B with addition to skimmed milk and sucrose generates better composition than that by inoculum A, particularly on total solids, total sugars, and reducing sugars, on the other hand it tends to be same on folic acid and dissolved protein. Purification on both biomasses through MF membrane at room temperature, stirrer rotation speed 300 rpm and TMP 20 and 40 psia for 30 minutes yields retentate (concentrate) and permeate, respectively, as seen in table 1. Physical properties of retentate (concentrate) is thick and turbid suspension, while permeate is white clear liquid and yellow clear liquid degrading with suitable color from each biomass. All of MF membrane techniques result concentrate with better composition compared to permeate. This matter is related to performance of MF technique, in which MF membrane (pore size 0.15 µm) will selectively retain
components with particle size > 0.15 µm either completely or partially on the surface of membrane known as retentate (concentrate), meanwhile components with particle size < 0.15 µm are permitted to pass freely across membrane area expressed as permeate (flux), although ‘fouling’ has an important role in separating each component. Fouling is a term generally used to describe the accumulation of various foulants, such as colloidal particles, macromolecules (proteins, polysaccharides), salts, etc. present in fluid onto active membrane surface (external fouling). Meanwhile, cake formation occurs in interacting among operation condition (temperature, rotation speed, TMP), and type of material (property, particle size) so that fluid which do not enter the pores, stack and form a cake layer on the membrane surface or sealed pores via intermolecular bonding and hydrophobic interactions [18-20]. The result of purification on biomass demonstrates successfully MF membrane performance on all components in biomass because of more retained components in retentate (concentrate) compared to passing through membrane as permeate. Folic acid, total solids and reducing sugars are components produced in retentate (concentrate) and permeate higher compared to feed. This matter caused by interaction between rotation speed and TMP is able to extract and separate until it can detected in spectrophotometry UV-vis [14], gravimetric and Somogyi Nelson methods [13]. MF membrane technique generates folic acid in biomass concentrate A and B 194.48 and 246.96 µg/mL, and permeate 10.37 dan 52.72 µg/mL, respectively, meanwhile other components indicates difference in both biomasses, particularly on total solids and dissolved protein. In this condition, MF membrane technique was able to separate components in biomass concentrate A, such as folic acid 191.92% (1.9 fold), total solids 28.4 %, dissolved protein 14.44 %, total sugars 20.26%, reducing sugars 223.53% (2.2 fold), and total acids 100% (1 fold), whereas in biomass concentrate A and biomass concentrate B contain folic acid 187.84% (1.88 fold), total solids 117.71% (1.18 fold), dissolved protein 84%, total sugars 32.43%, reducing sugars 110.87% (1.1 fold), and total acids 100% (1 fold) compared to concentrations for each component in biomass feed A and biomass feed B. Meanwhile, figure 1 (a-f) displayed biomass A, retentate, and permeate as a result of MF on biomass A at 300 rpm and TMP 20 psia, biomass B, retentate, and permeate as a result of MF on biomass B at 300 rpm and TMP 40 psia, respectively.

Table 1. Characteristic of biomass.

| Kind of Biomass | Kind of material | Folic acid (µg/mL) | Total solids (%) | Dissolved protein (mg/mL) | Total sugars (mg/mL) | Reducing sugars (mg/mL) | Total acids (%) |
|-----------------|-----------------|-------------------|------------------|--------------------------|----------------------|------------------------|----------------|
| White corn (A)  | Feed            | 66.62             | 27.08            | 0.90                     | 1.925                | 0.17                   | 0.10           |
|                 | Concentrate**   | 194.48            | 7.69             | 0.13                     | 0.390                | 0.38                   | 0.10           |
|                 | Permeate**      | 10.37             | 5.00             | 0.04                     | 0.050                | 0.14                   | 0.10           |
| Yellow corn (B) | Feed            | 86.45             | 54.30            | 0.25                     | 21.860               | 22.64                  | 0.19           |
|                 | Concentrate***  | 246.96            | 46.13            | 0.21                     | 7.090                | 20.42                  | 0.19           |
|                 | Permeate***     | 52.72             | 28.75            | 0.13                     | 3.910                | 2.19                   | 0.10           |

Legend: *A: biomass of nixtamalized white corn using inoculum of *B. brevis 40% without skimmed milk and sucrose, fermentation at 40 °C for 24 hours; B: biomass nixtamalized yellow corn using inoculum of *B. brevis 40%, with skimmed milk (10%) and sucrose (10%), fermentation at 40 °C for 24 hours; **result of MF at rotation speed 300 rpm, TMP 20 psia; ***result of MF at rotation speed 300 rpm, TMP 40 psia.
Figure 1. (a) Biomass A, (b) retentate and (c) permeate as a result of MF on biomass A at 300 rpm, TMP 20 psia, (d) biomass B, (e) retentate and (f) permeate as a result of MF on biomass B at 300 rpm, TMP 40 psia.

4.2. Effect of drying process on composition

Drying biomass concentrate A and biomass concentrate B at 50 °C for 36 hours produce powder with degraded white and less pink color, and degraded yellow and less brownish, as seen in Figure 2 (a) and (b), and its composition shows in difference, as shown in Table 2. All of treatments, drying process increases concentration of folic acid (FA) on both type of biomass concentrate powder caused by evaporating water mass.

![Figure 2. (a) biomass concentrate powder A and (b) biomass concentrate powder B.](image)

Table 2. Compositions of biomass concentrate powder A and biomass concentrate powder B.

| Kind of powder * | Kind of Component |
|------------------|-------------------|
|                  | Folic acid (µg/mL) | Dissolved protein (mg/mL) | Total sugar (mg/mL) | Reducing sugar (mg/mL) | Total solid (%) | Total acid (%) |
| Biomass concentrate A | 565.35 | 0.19 | 2.540 | 1.718 | 96.60 | 0.29 |
| Biomass concentrate B | 614.92 | 0.30 | 59.00 | 59.300 | 96.76 | 0.41 |

Legend: *result of drying cabinet at 50 °C for 24 – 36 hours.

All of treatments, drying process increases concentration of folic acid on both type of biomass concentrate powder caused by evaporating water mass. Difference in this result is possibility caused by concentration of folic acid in different initial material before drying. Folic acid in higher biomass concentrate B (246.96 µg/mL) than that in biomass concentrate A (194.48 µg/mL). On total solids in higher biomass concentrate B (46.13%) compared to total solids biomass concentrate A (7.69%) will cause faster evaporator. This matter causes folic acid in biomass concentrate powder B achieved higher compared to biomass concentrate powder A. Total solids are accumulation of all soluble and non-soluble according to Gravimetric method [13]. This drying method raises components in biomass concentrate powder A, such as folic acid 190.52% (1.9 fold), dissolved protein 551.28% (5.51 fold), total sugars 46.15 %, reducing sugars 78 %, total solids 115.67% (1.16 fold), total acids 190% (1.9 fold), and components in biomass concentrate powder B, such as folic acid 148.99% (1.5 fold), dissolved protein...
73.216% (7.32 fold), total sugars 30%, reducing sugars 190.40% (1.9 fold), total solids 109.75% (1.1 fold), and total acids 115.79% (1.16 fold) compared to concentration from each component in concentrate A and concentrate B before drying.

### 4.3. Identification of folic acid monomer in biomass concentrate powder

Identification on folic acid monomer in standard folic acid with mass spectra m/z ranging 439 – 448 shows monomer domination with molecular weight (MW) 442.32, 442.60 and 442.93 Dalton (Da), and relative intensity 100%, 41.63%, and 27.5%. Folic acid had been known MW 441 Da. Via LC-MS method had been known that a compound indicated by difference in MW is possibility 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. Operation condition of LC-MS is injection volume 5 μL, flow rate 0.2 mL/minute at the methanol-to-water ratio of 80 : 20 using column C-8 (15 mm x 2 mm) [15]. Mass spectra on biomass concentrate powder A and biomass concentrate powder B at m/z from 441.0 to 443.0 and from 441.81 to 444.0 generate 6 and 7 folic acid monomer dominated by folic acid monomer with MW 442.72 and 442.07 Da. and relative intensity 100 % and 75%, as seen in figure 3 (a-c). All folic acid monomers on three materials are displayed in table 3.

![Figure 3](image-url)

**Figure 3.** (a) Mass spectra of standard folic acid, (b) mass spectra of biomass concentrate powder A and, (c) mass spectra of biomass concentrate powder B

| Index | Centroid Mass | Relative Intensity (%) | Are |
|-------|---------------|------------------------|-----|
| Biomass concentrate A | 3165 | 442.07 | 8.59 | 7.68 |
| 3166 | 442.29 | 3.43 | 0.83 |
| 3167 | 442.49 | 3.44 | 1.86 |
| 3168 | 442.65 | 6.87 | 0.83 |
| 3169 | 442.74 | 6.87 | 1.00 |
| 3170 | 442.94 | 3.44 | 2.50 |
| Biomass concentrate B | 2939 | 442.06 | 4.04 | 3.55 |
| 2940 | 442.24 | 2.02 | 4.00 |
| 2941 | 442.45 | 3.03 | 2.75 |
| 2942 | 442.53 | 2.02 | 1.00 |
| 2943 | 442.72 | 3.03 | 6.01 |
| 2944 | 442.85 | 1.01 | 1.00 |
| 2945 | 442.98 | 2.02 | 1.50 |

### 4.4. Particle size distribution

Biomass concentrate powder A and biomass concentrate powder B are the result of drying on biomass concentrates A and B from process of purifying by means of MF membrane which are white to reddish white color and brownish yellow. A series of process, that had been followed on both type of materials affects possibility on particle size and particle distribution. Table 4 showed particle distribution on both
type of concentrate powders, in which biomass concentrate powder A (nixtamalized white corn concentrate by e 461.0 nm with dispersed particle (Particle Index) 0.653.

**Table 4.** Characteristic of folate particle in concentrate powder as a result of MF on white corn and yellow corn fermented by Bifidobacterium brevis.

| Kind of material                                      | Particle Distribution (nm) |
|-------------------------------------------------------|----------------------------|
|                                                        | Z-Average (nm)* | PI** |
| Concentrate powder of fermented white corn (Biomass A) | 1115.1          | 0.827 |
| Concentrate powder of fermented yellow corn (Biomass B)| 461.0           | 0.653 |

Legend: *Diameter of nano particles; **dispersed particles.

This difference is possibility caused by biomass concentrate powder A which is without enriching skimmed milk and sucrose so that suspension is dominated by corn granula, meanwhile on biomass concentrate powder B is conducted enriching skimmed milk and sucrose so that texture and smoother. Interaction amongst processes of homogenization (8000 rpm, 30 minutes), microfiltration membrane and drying affect on particles size of concentrate powder possibility caused by dispersed particle and particle size. In analysis of particles through DLS system, Z-Average indicated diameter size (Ø), Particle Indexes (PI) and particle distribution. Value of PI > 1 indicated that material has broad range of distribution or not homogeneous, while value of PI < 1 showed homogeneous product [21]. Both type of biomasses with values of PI < 1 displays uniform particle size.

Particle distribution in biomass concentrate powder A gave subsequently diameter size (Ø) in the range 100 – 200 nm (< 1000 nm) at frequency (dispersed particles) ranging 0 – 55%, Ø in the range 110 – 1500 nm at frequency (dispersed particles) ranging 0 – 30% and Ø in the range 1100 – 2000 nm (< 10000 nm) at frequency (dispersed particles) ranging 0 – 11%. The whole biomass concentrate powders A contain particles with size in the range 100 – 1500 nm and dispersed particles to 25%, as seen in figure 4 (a). Different trend seems at particle distribution in biomass concentrate powder B, in which it is yielded particle with Ø in the range 110 – 175 nm (< 1000 nm) at frequency (dispersed particles) ranging 0 – 2% or Ø in the range 110 – 6000 nm (< 10000 nm) at frequency (dispersed particles) > 55% and Ø in the range 4000 – 7000 nm (< 10000 nm) at frequency (dispersed particles) ranging 0 – 45%. In other words, average particles size in biomass concentrate powder B in the range 110 – 7000 nm at frequency (dispersed particles) ranging 0 to > 55%, as in shown in Figure 4 (b).

![Figure 4](image.png)

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5. Conclusion

A result of experiment activities showed that interaction among three kind of treatments affected on composition, particularly folic acid, domination of folic acid and particle size distribution. Biomass concentrate powder A and B are generated by means of MF membrane at room temperature, stirrer rotation speed 300 rpm and TMP 20 psia and 40 psia for 30 minutes and drying at 50 °C for 24 – 48
hours, respectively. In this condition, biomass concentrate powder A and B gave compositions of folic acid 565.35 μg/mL and 614.92 μg/mL, total solids 96.60 mg/mL and 96.76 mg/mL, dissolved protein 0.19 mg/mL and 0.30 mg/mL, reducing sugars 1.718 mg/mL and 59.300 mg/mL, total sugars 2.54 mg/mL and 59.00 mg/mL, and total acids 0.29% and 0.41%, respectively. Drying system increases folic acid in biomass concentrate powder A and B 190.52% (1.9 times) and 148.99% (1.5 times), dissolved protein 551.28% (5.51 times) and 732.16% (7.32 times), total sugars 115.67% (1.16 times) and 109.75% (1.1 times), and total acids 190% (1.9 times) and 115.79% (1.16 times) compared to concentration of each component in concentrates of A and B prior to process drying. In this condition, biomass concentrate powder A and B demonstrated domination of folic acid monomer with molecular weight (MW) 442.07 Da. and 442.72 Da. at relative intensity 75% and 100% with particle size 1115.1 nm and 461.0 nm and Index Partikel 0.827 and 0.653.

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