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Formulation of Emulsion of Soybean (*Glycinia soyae* L.) Tempeh and Fermented Spinach (*Amaranthus* sp.) using Combination of Gelatin and CMC as Thickener

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Abstract

Mixture of soybean (*Glycinia soyae* L.) tempeh paste and spinach (*Amaranthus* sp.) were fermented by Kombucha culture produces potential source of natural folic acid. The aim of study is to find out the best ratio of gelatin and CMC as thickener in emulsification to prepare supplement of natural folic acid. Emulsification process was conducted by adding gelatin and CMC at ratio of 0:0, 0.1:2, 0.2:4, 0.3:6, 0.4:8, and 0.5:10 (g/g) in mixture of fermented spinach concentration of 2.5, 5.0, 7.5, and 10.0% (w/w in soybean tempeh) and soybean tempeh. The best drying treatment was achieved at 50°C for 24 h. The optimum treatment was obtained at combination of gelatin-CMC ratio of 0.4:8 (g/100 g in mixture paste) and fermented spinach 2.5%. Identification with mass spectra had been identified 6 folic acid monomer dominated by folic acid monomer with molecular weight (M+1) 442.87 and 442.66 Dalton (Da.) with relative intensity 100%. Paste and powder of supplement displayed distribution of particle size 1,745.1 and 984.1 nm with particle indexes of 1.124 and 1.121.

Keywords: emulsification, folic acid, gelatin, ratio, spinach, tempeh

Formulasi Emulsi Campuran Tempe kedelai (*Glycinia soyae* L.) dan Bayam (*Amaranthus* sp.) Terfermentasi Menggunakan Gelatin dan CMC sebagai Pengental

Abstrak

Campuran pasta tempe kedelai (*Glycinia soyae* L.) dan bayam (*Amaranthus* sp) terfermentasi oleh kultur Kombucha menghasilkan sumber asam folat alami. Penelitian bertujuan untuk mengetahui rasio gelatin dan CMC terbaik sebagai pengental dalam emulsifikasi tempe dan bayam terfermentasi untuk membuat asam folat alami. Proses emulsifikasi dilakukan oleh penambahan gelatin dan CMC pada rasio 0:0, 0.1:2, 0.2:4, 0.3:6, 0.4:8 dan 0.5:10 (g/g) pada campuran bayam terfermentasi dengan konsentrasi 2.5, 5, 7.5 dan 10% (b/b tempe kedelai), dan tempe kedelai. Perlakuan pengeringan terbaik dilakukan pada 50°C selama 24 jam. Perlakuan optimum dicapai dengan kombinasi gelatin dan CMC pada rasio 0.4:8 (g/100 g pasta campuran) dan bayam terfermentasi 2.5%. Identifikasi dengan spektra massa ditemukan 6 monomer asam folat yang didominasi oleh monomer asam folat berberat molekul (M+1) 442,87 dan 442.66 Da. dengan intesitas relatif 100 %. Distribusi partikel pasta dan sebuk suplemen berukuran masing-masing 1745,1 dan 984,1 nm dengan indeks partikel 1,124 dan 1,121.

Kata Kunci: asam folat, bayam, emulsifikasi, gelatin, rasio, tempe

1. Introduction

Processes of emulsification and drying on mixture pasta of soybean (*Glycinia soyae* L.) tempeh as a result of fermentation by *Rhizopus oligosporus* strain C_1 and spinach (Amaranthus sp.) fermented by Kombucha culture is an important effort and a novelty process to get natural folic acid.[1] Folic acid (folate, folacin, vitamin B9, pteroyl-L-glutamic acid, pteroyl-L-glutamate, pteroyl monoglutamic acid) is an important micronutrients needed by pregnant women, children in the growth period, and older men and women.[2] Chemical structure of folic acid consist of a pteridine ring, a para-aminobenzoate (p-ABA), and a g-linked tail with one or more L-glutamates.[3]

As precursor in nucleotide synthesis of remethylation to homocysteine, folic acid has main role at both cells fission and growth, particularly to produce red blood cells in order to prevent anemia and prevent emergence of Neural tube defects (NTDs) on infants. By consumming or intaking 0.4 – 8 mg (400 – 800 ug) of folic acid daily to women prior to and during pregnancy, risk of emergence of NTDs on infants can be lowered to 80 %. In order to prevent the occurrence of Neural tube defects (NTDs) and neural system defects, women planning pregnant should consume folic acid sufficiently for minimal 4 months before pregnant.[2,4]

Activity of protease enzyme of *Rhizopus oligosporus* strain-C1 in degrading substrate components in tempeh protein affects on formation of folic acid. On spinach fermented by Kombucha culture, degrading the whole components to folic acid is caused by activities of dihydrofolate synthase (DHFS) and polyglutamate synthase (FPGS) enzymes to form dihydrofolate and polyglutamylated folates, respectively. A form of folic acid derivative[5] in this process occur only in plant and microbes. Kombucha cultures contain acetic acid bacteria (*Acetobacter xylinum*), yeast (*S. cereviseae, S. ludwigii, Zygosaccharomyces* sp.) and many types of fungi (*Torolupsis* sp.),[6] degrading polyphenol and amino acids.[7]

Supplement of folic acid yielded from mixture of soybeans tempeh and fermented spinach through emulsification with mixture of gelatin and carboxy methyl cellulose (CMC) are expected to produce paste and powder with better composition of folic acid. CMC is a cellulose derivative with carboxymethyl groups ($-CH_2-COOH$) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone. CMC with a degree of substitution (DS) of 0.7. CMC has good binding, thickening, and stabilizing properties on fat, eases to solve in cold and hot water, saves consumption according to standard use with concentration of < 8% (product weight).[8] Gelatin is selected because of contribution on protein and gel phase property so that combination between gelatin and CMC enables to yield mixture paste through better binding system of folic acid. Folic acid has property, such as sensitif on light, oxygen, high temperature, and mechanical treatment so that process treatment in order to keep stabilizing property of folic acid is an important process. Emulsification process by homogenizing at 8000 rpm for 15 – 30 minutes enables to get mixture paste with small particle size in order to facilitate adsorption of folic acid in stomach.

Mixture paste of soybeans tempeh and fermented spinach at the best ratio is achieved by adding thickener from mixture of gelatin and CMC at appropriate ratio, and their interactions between emulsification and drying will yield powder of natural folic acid.

The objective of this experiment activity is to find out at gelatin-to-CMC ratio as binder and thickener added to soybeans tempeh and fermented spinach to get mixture pasta of natural folic acid on composition, identification of folic acid monomer, and characteristic of distribution of particle size as supplement of natural folic acid.

2. Methods

2.1. Equipments

Main equipments utilized in this experiment activity were balance (Fujitsu, Japan), blanching, blender (National, local), sieve of 60 and 80 mesh (Retsch, Germany), autoclave (CHENG YI, LS – 50 L, China), a series of microbiology equipments, a series of fermentation equipments, homogenizer (Ultra-Turrax, Ika Labortechnik, T50, Jane & Kunkel, Germany), system of laminar flow chamber (local), incubator (local), and a series of nixtamalization process in laboratory scale. Main instruments for analysis were UV-vis Spectrophotometer (Model RF-550, Shimadzu, Japan), and Liquid Chromatography-tandem Mass Spectrometry (LC-MS) (Mariner Biospectrometry) with LC (Hitachi L 6200), and Particle Size Analyzer (PSA) (Horiba Nano Partica-Backman).

2.2. Materials

Main materials used in this experiment activity were soybeans and fresh spinach purchased from a local market, *Rhizopus oligosporus* strain C1, Kombucha culture (Research Center for Chemistry – LIPI), gelatin (Brataco), CMC (Brataco), and sucrose (local). All the chemicals used in this process and analysis procured locally were reagent grade.

2.3. Experimental design

A series of experiment steps were conducted through fermentation of soybeans by inoculum of *Rhizopus oligosporus* strain C1 and fermentation of spinach using spinach inoculum from Kombucha stock culture, mixing on mixture pasta of fermented spinach and soybeans tempeh at concentration of 2.5, 5.0, 7.5 and 10% (w/w in soybeans tempeh), gelatin-to-CMC ratio of 0 : 0, 0.1 : 2, 0.2 : 4, 0.3 : 6, 0.4 : 8 and 0.5 : 10 (g), and homogenizing 8000 rpm for 30 minutes so that it is get mixture pasta of soybeans tempeh and fermented spinach. Based on folic acid, the best result of treatment is further dried using cabinet dryer at 50°C for 24 hours, size reduction and sieving through 60 – 80 mesh so that it is generated mixture powder of soybeans tempeh and fermented spinach as supplement preparate of natural folic acid. Analysis was carried out on dissolved protein (Lowry method), total solids (Gravimetric method), reducing sugars (Somogyi-Nelson), total sugars (Phenol Sulfate) and folic acid (spectrofotometry UV-vis).[9] Identification on folic acid monomer was performed by means of LC-MS with LC,[10] particle size distribution was determined by Particle Size Analysis (PSA).[11]

2.4. Details of procedure

2.4.1. Fermentation process of mung beans (tempeh)

A number of soybeans were sorted, cleaned washed, steamed for 30 – 45 minutes, cooled, and soaked in water (pH 5) overnight. Further day, soaked soybeans were peeled, washed, allowed, and inoculated by inoculum of *Rhizopus oligosporus* strain C1 at concentration 0.2% (w/w), covered in perforated plastic, and incubated at room temperature (28 – 30 °C) for 24 – 36 hours until it is seemed micelia fulfilling soybeans surface.[1]

2.4.2. Fermentation process of spinach

Broccoli was blanched at 80 °C for 5 minutes, pulverized by adding water at spinach-to-water of 1 part : 4 parts to get pulp, and filtered through 60 mesh sieve. Filtrate (suspension) was poured with 15% (v/w in spinach suspension) broccoli inoculum and 10% (w/w in spinach pulp) sucrose, and stored in closed container followed by aeration (cassa cloth) at room temperature (28 – 30 °C) in dark room for 3 days. The whole works were carried out aseptically. Biomass produced was fermented spinach suspension.

2.4.3. Mixing, homogenizing, drying, size reduction and sieving

Initial process was performed by mixing and homogenizing mixture pasta of fermented spinach in soybeans tempeh at concentration 2.5, 5.0, 7.5 and 10% (w/w in soybeans tempeh) at 8000 rpm for 30 minutes. On each treatment was added mixture of gelatin into CMC at concentrations of 0, 0.1, 0.2, 0.3 and 0.5 (w/w in CMC), and homogenized at 8000 rpm for 30 minutes in order to produce mixture pasta of soybeans tempeh and fermented spinach. Based folic acid, on the best treatment was conducted a drying on mixture pasta of soybeans tempeh

Table 1. Composition of materials

Type of materials	Types of components				
	Folic acid (µg/mL)	Dissolved protein (mg/mL)	Total solids (%)	Reducing sugars (mg/mL)	Total sugars (mg/mL)
Soybeans tempeh	217.71	0.46	37.81	16.31	134.41
Fermented spinach*	96.58	0.16	4.47	64.91	34.91

Legend: *spinach fermented for 3 days

and fermented spinach at 50 °C for 24 hours, size reduction, and sieving through 80 mesh so that it is generated mixture powder of soybeans tempeh and fermented spinach as supplement preparate of natural folic acid.

3. Results

3.1. Characteristics of materials

Table 1 showed characteristic of materials in preparation of supplement of natural folic acid, meanwhile Table 2 showed composition of recovery of paste supplement produced from emulsification of materials.

3.2. Identification of folic acid

Figure 1 demonstrated mass spectra of paste supplement and powder supplement at optimum condition of emulsification, respectively.

3.3. Distribution of particle size

Characteristic of distribution of paste particles and supplement powder of natural folic acid was displayed in Table 3, whereas Figure 2 demonstrate relationship between particle size and frequency on particles distribution in paste supplement and supplement powder of folic acid at optimum condition of emulsification.

4. Discussions

4.1. Characteristics of materials and compotitions of suplement pastes

Main materials used in preparation of supplement of natural folic acid were mixture of soybeans tempeh and fermented spinach as a result of fermentation by inoculum of *R. oligosporus* strain C1 and Kombucha stock culture. Fermentation of soybeans by inoculum of *Rhizopus oligosporus* C1 produces tempeh with maturity, micelia spreading and the best texture achieved at room temperature for 24 hours faster than fermentation of soybeans by mixture of *Rhizopus sp.* in general. Use of *R. oligosporus* strain-C1 does not take place competitor of fungi in using *Rhizopus sp.* mixture compared with inoculum in general which are various types of *Rhizopus sp.* species.[1] During fermentation took place a physically change and composition of tempeh nutrition, particularly on folic acid related with enzyme activity of *R. oligosporus* strain-C1, such as protease and amylase enzymes. Composition of soybeans tempeh indicates higher concentrations of folic acid, dissolved protein, total solids and total sugars, and lower

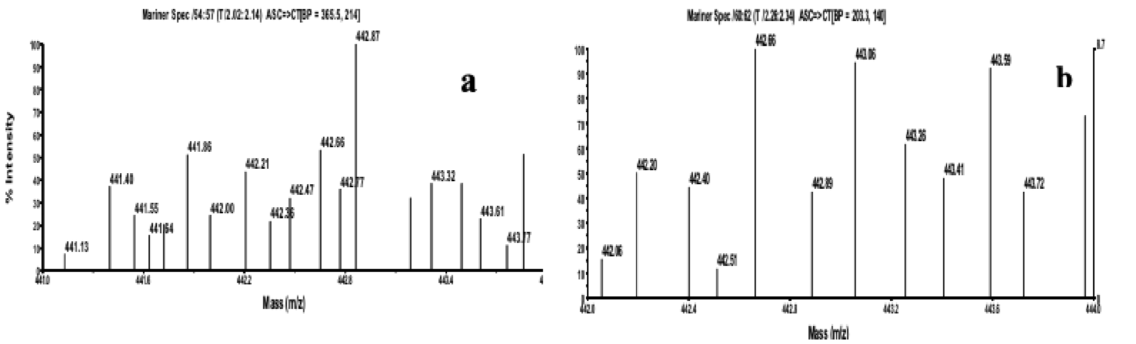

Figure 1. (a) mass spectras from supplement paste and (b) supplement powder with treatment of fermented spinach 2.5% (w/w, soybean tempeh), and gelatin and CMC at ratio of 0.4 : 8 (g)

Table 2. Compotitions of suplement pastes

Concentration of Fermented spinach (% w/w soy bean tempeh)	Ratio Gelatin:CMC	Kind of component				
		Folic acid (µg/mL)	Dissolved protein (mg/mL)	Total solid (%)	Total sugar (mg/mL)	Reducing sugar (mg/mL)
2.5	0:0	246.67	1.38	13.40	28.51	20.19
	0.1:2	326.98	1.72	14.11	23.22	14.41
	0.2:4	181.85	3.18	17.97	57.79	27.87
	0.3:6	456.89	3.03	18.10	45.11	16.42
	0.4:8	769.67	3.65	20.70	42.90	15.92
	0.5:10	577.31	5.21	20.4	57.83	30.85
5	0:0	285.69	1.39	10.34	28.51	26.35
	0.1:2	239.85	1.61	10.39	23.22	12.17
	0.2:4	336.63	2.86	14.56	57.79	26.01
	0.3:6	511.60	3.46	17.12	45.11	20.60
	0.4:8	426.90	3.42	20.13	42.90	11.12
	0.5:10	640.38	5.29	20.69	57.83	28.67
7.5	0:0	268.69	0.70	10.23	36.56	19.84
	0.1:2	251.29	1.32	8.44	21.91	10.91
	0.2:4	299.42	3.04	13.80	40.58	27.21
	0.3:6	572.35	3.658	14.80	47.76	15.35
	0.4:8	594.23	3.29	18.78	46.57	12.28
	0.5:10	380.56	4.65	19.35	45.13	18.74
10	0:0	317.08	0.84	9.53	29.33	26.44
	0.1:2	328.94	1.26	12.31	24.99	9.70
	0.2:4	355.08	2.51	13.83	33.70	20.60
	0.3:6	344.62	3.45	14.05	45.95	13.05
	0.4:8	502.48	3.47	18.21	58.97	10.80
	0.5:10	663.51	4.08	21.41	39.23	16.75

concentration of reducing sugars compared with fermented spinach, as shown in Table 1. This matter is not only caused by difference in raw material, but also by enzyme activities in degrading materials and fermentation system. High concentration of reducing sugars occur possibility because not all sucrose is used in metabolism of Kombucha culture, in which it will be formed from reducing sugars so that it remains sugars in biomass. Spinach fermentation for days is appropriate period with fermentation optimization on folic acid. This matter is not only influenced by initial concentration of materials, particularly chlorophyll, but also by proteolytic activity of Kombucha culture, time and fermentation temperature. It had been indicated that proteolytic activity of Kombucha culture on substrate of spinach is higher (0.0964 µ/mg) compared to other substrate (broccoli) (0,0714 µ/mg), which causes ability to degrade better dissolved protein. Chlorophyll is source of nitrogen used to form peptides and amino acids through photosynthesis. It had been known that initial concentration of chlorophyll in spinach is higher (10.2642%) compared to other green vegetables, such as broccoli (3.896%).

Effect of emulsification and homogenization on composition of supplement pasta shown in Table 2. Folic acid and dissolved protein are one of unity compounds because of folic acid structured on pteridine ring, para-aminobenzoate (p-ABA), and g-linked tail with one or more L-glutamates.[3] Emulsification process

Table 3. Characteristic of pasta and powder material for natural folic acid supplement

Kind of of material*	Distribution of nano-folate particles (nm)	
	Z-Average (nm)**	PI***
Pasta of mixed fermented spinach and soybean tempeh	1745.1	1.124
Powder of mixed fermented spinach and soybean tempeh	984.1	1.121

Legend: *from treatment fermented spinach 2.5% (w/w in soybean tempeh) with gelatin 0.4% (w/w in CMC); diameter of nano particles ; **dispersed particles (Particle Index).

of mixture of fermented spinach and soybeans tempeh at gelatin-to-CMC ratio becoming more and more increase generates supplement pasta with composition of folic acid and dissolved protein. Emulsification rate tends to fluctuate, however it increases relating with gelatin-to-CMC ratio, in which it is took place optimization of folic acid (769.67 µg/mL) with treatment combination at gelatin-to-CMC ratio of 0.4 : 8 on mixture pasta of fermented spinach 2.5% (w/w in tempeh) followed by declining folic acid at higher gelatin-to-CMC ratio of (0.5 : 10). On dissolved protein, this increase of gelatin-to-CMC ratio reaches to the highest gelatin-to-CMC ratio (5.29 mg/mL) and it is achieved at gelatin-to-CMC ratio of 0.5 : 10 and mixture pasta of fermented spinach 5% (w/w in tempeh). Fluctuated folic acid is possibility caused by contribution of CMC concentration becoming more and more high so that it is able to cover folic acid from denaturation due to mechanical force of homogenizer. CMC will trap suspended folic acid in matrix in order to stable due to gravitation force. In other words, cellulose content binds tape confirmation to form hydrogen bridge with other components and catch water mass as hydrocolloid.[8,12] Gelatin in this context has ability to thick pasta and contribute on composition, particularly protein. This matter seems with increasing dissolved protein to the highest gelatin-to CMC ratio. Compared to control treatment (gelatin-to-CMC ratio of 0 : 0), optimum treatment increases folic acid from 246.67 µg/mL to 1.393 mg/mL (212% or 2.12 folds) and dissolved protein from 769.67 µg/mL to 5.29 mg/mL (280.57% or 2.86 folds).

Emulsification process on fermented spinach and soybeans tempeh followed by adding gelatin and CMC at high ratio produces supplement pasta with high content of total sugars. Increasing total sugars is not only possibility caused by increase fermented spinach on soybeans tempeh, but also by ability of gelatin and CMC in order to bind these components on emulsification process. Fermented spinach remains total sugars, which is not used by microbes to its metabolisms during fermentation.[13] Gelatin and CMC does not contribute on total sugars. Optimization of emulsification process on total sugars (58.98 mg/mL) is achieved at treatment of fermented spinach at concentration of 10% (w/w in tempeh) followed by adding gelatin-to-CMC ratio of 0.4 : 8 (gram). Compared with control treatment (gelatin-to-CMC ratio of 0 : 0), this optimum treatment increase total sugars from 29.33 mg/mL to 58.98 mg/mL (101.09%).

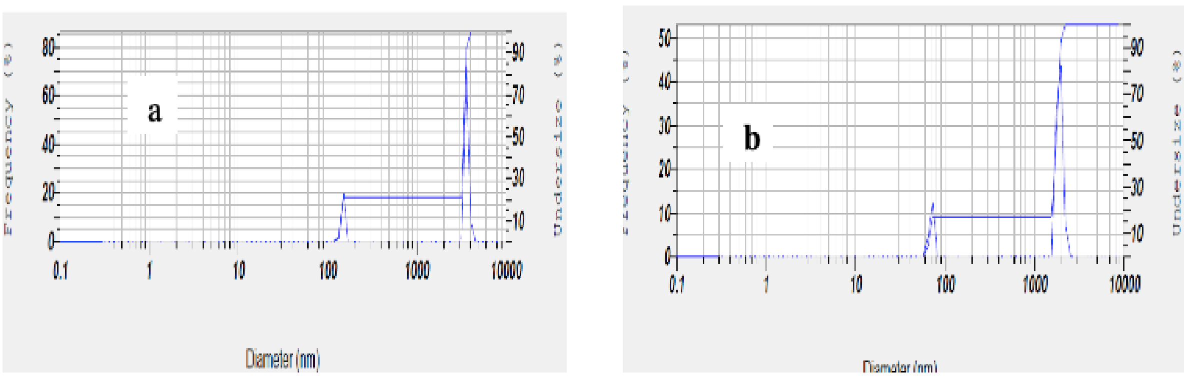

Figure 2. Relationship between particle size and frequency on particles distribution in (a) paste supplement and (b) powder supplement of folic acid at optimum condition of emulsification

On reducing sugars, interaction between gelatin-to-CMC ratio and fermented spinach concentration in soybeans tempeh tends to generate fluctuated. Reducing sugars is result of metabolite produced during fermentation process of spinach by Kombucha culture.[15] It had been seemed that it took place a decrease of reducing sugars relating with increasing fermented spinach concentration in each gelatin-to-CMC ratio treatment. On fermented spinach concentrations 2.5, 5 and 7.5% took place increasing reducing sugars indicating that both gelatin and CMC are able to bind reducing sugars, however it is not able to bind fermented spinach with higher concentration (10%). Dropping reducing sugars is possibility caused by homogenization system (8000 rpm, 30 minutes) lysing a part of reducing sugars, which is not detected according to Nelson-Somogyi.[14] Optimization of emulsification process on reducing sugars (30.85 mg/mL) is reached at treatment of fermented spinach at concentration 2.5% (w/w in tempeh) by adding gelatin to CMC at ratio of 0.5 : 10 (gram). Compared with control treatment (gelatin-to-CMC ratio of 0 : 0), this optimum treatment increases reducing sugars from 20.19 mg/mL to 30.85 mg/mL (57.80%). Emulsification process increases also total solids in supplement pasta relating with increasing fermented spinach concentration through gelatin-to-CMC ratio. Total solids is accumulation of all soluble and insoluble components in water. Increasing total solids is caused by contribution of CMC to fermented spinach concentration of 7.5%, however it is took place a drop of total solids at the highest concentration (10%) for all gelatin-toCMC ratios. Fluctuated total solids is enabled to be occurred due to interaction between homogenization and materials causing occurrence of denaturation, lysis or diluting materials. Optimization of emulsification process on total solids (21.41%) is reached by treatment on fermented spinach at concentration 10% (w/w in tempeh) and gelatin-to-CMC ratio of 0.4 : 8 (gram). Compared to control treatment (gelatin-to-CMC ratio of 0 : 0), this optimum treatment increases total solids from 9.53% to 21.41% (124.66% or 1.2 folds).

4.2. Optimum condition of emulsification process

From review of effect of emulsification process on composition of supplement pasta, based on the highest recovery of folic acid, the best process condition was achieved with treatment combination at gelatin-to-CMC ratio of 0.4: 8 and concentration of fermented spinach 2.5% (w/w in soybeans tempeh). This process condition is produced supplement pasta with compositions of folic acid 769.67 µg/mL, dissolved protein 3.65 mg/mL, total sugars 41.11 mg/mL, reducing sugars 15.92 mg/mL, and total solids 20.70%. Drying process at 50°C yields supplement powder with compositions of folic acid 615.31 µg/mL, dissolved protein 10.72 mg/mL, total solids 91.37 %, total sugars 15.59 mg/mL, and reducing sugars 92.80 mg/mL so that it decreases folic acid 20.05% and total sugars 62.1%, however it increases total solids 341.4% (3.41 folds), dissolved protein 193.7% (1.9 folds), and reducing sugars 482.80% (4.82 folds) compared with each component in supplement pasta prior to drying process.

4.3. Identification of folic acid

Pasta and supplement powder from treatment of fermented spinach 2.5% (w/w in soybeans tempeh), and gelatin dan CMC ratio of 0.4 : 8 (g) showed size and particle index are summarized in Table 3. Difference in size and particle index are possibility caused by difference in processes related. In this preparation process on supplement pasta, applying homogenizer at 8000 rpm for 30 minutes causes its occurrence of size reduction of particles in order to yield supplement pasta with particle size of 1,745.1 nm, and particle size become more and more small by drying process due to its occurrence of water mass evaporation (984.1 nm). Difference in particle size is an important reference in preparing both supplement and pharmaceutical products because of direct related to digestive system. Processes related to pasta or supplement powder affects on particle size, which can be identified from total solids accumulating the whole soluble and insoluble components according to Gravimetric method.[15] On dispersed particle index according to DLS method, particle size becoming more and more small will lower particle index or in other words dispersed particles are more uniform and homogen. Both type of materials have particle index larger than 1 (one) displaying insufficient homogen particle size, while particle index is smaller 1 (one) demonstrating uniform particle size.[11] This matter is enabled by size reduction or insufficient optimal sieving. Different matter seems in Figure 2a, in which particle size distribution generates particles with diameter (Ø) size range of 100 – 1300 nm (> 1000 nm) at frequency (dispersed particles) 20% and particle with Ø > 1500 nm at frequency 85%. All pasta products results particle with Ø range of 100 – 2000 nm at frequency 85%, whereas supplement powder yields particle with Ø range of 80 – 1300 nm at frequency 10% and it increases to frequency > 40% with Ø range 1300 – 2000 nm, as showed in Figure 2b.

4.4. Distribution of particle size

Pasta and supplement powder from treatment of fermented spinach 2.5% (w/w in soybeans tempeh), and gelatin dan CMC ratio of 0.4 : 8 (g) showed particle distribution, as shown in Figure 2a and Figure 2b, while size and particle index are summarized in Table 3. Difference in size and particle index are possibility caused by difference in processes related. In this preparation process on supplement pasta, applying homogenizer at 8000 rpm for 30 minutes causes its occurrence of size reduction of particles in order to yield supplement pasta with particle size of 1,745.1 nm, and particle size become more and more small by drying process due to its occurrence of water mass evaporation (984.1 nm). Difference in particle size is an important reference in preparing both supplement and pharmaceutical products because of direct related to digestive system. Processes related to pasta or supplement powder affects on particle size, which can be identified from total solids accumulating the whole soluble and insoluble components according to Gravimetric method.[15] On dispersed particle index according to DLS method, particle size becoming more and more small will lower particle index or in other words dispersed particles are more uniform and homogen. Both type of materials have particle index larger than 1 (one) displaying insufficient homogen particle size, while particle index is smaller 1 (one) demonstrating uniform particle size.[11] This matter is enabled by size reduction or insufficient optimal sieving. Different matter seems in Figure 2a, in which particle size distribution generates particles with diameter (Ø) size range of 100 – 1300 nm (> 1000 nm) at frequency (dispersed particles) 20% and particle with Ø > 1500 nm at frequency 85%. All pasta products results particle with Ø range of 100 – 2000 nm at frequency 85%, whereas supplement powder yields particle with Ø range of 80 – 1300 nm at frequency 10% and it increases to frequency > 40% with Ø range 1300 – 2000 nm, as showed in Figure 2b.

5. Conclusions

Optimization of emulsification process was achieved at combination between fermented spinach 2.5% (w/w, soy tempeh) at gelatin and CMC ratio of 0.4 : 8 (gram) producing supplement powder with folic acid 615.31 μg/mL, dissolved protein 10.72 mg/mL, total solids 91.37%, total sugars 15.59 mg/mL, and reducing sugars 92.80 mg/mL dominated by monomer of folic acid with molecular weight (MW) 442.66 Da. (M+1) with relative intensity 100% and distribution of particles size 984.1 nm with particle index 1.121.

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