Formulation of mixture powder as fortificant of natural folic acid in preparation of infant cream soup for complementary feeding

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Abstract. Mixtures of both nixtamalized yellow and white corn (Zea mays var. indentata), mung bean (Phaseolus radiatus) tempeh, and fermented broccoli (Brassica oleracea L.)/spinach (Amaranthus sp.) is a recovering fortificant of natural folic acid in formulation of infant cream soup as complementary feeding. The experiment activity aims to find out optimization of their formulation and volatiles compounds. The experiment activity was done by using four types of fortificant subsequently A, B, C and D with folic acid concentration in fortificants 0, 100, 200, 300, 400, and 500 µg/75 g in basic formula of cream soup. The result of experiment work showed that based on dissolved protein, formulation optimization was achieved at fortificant C with folic acid concentration of 600 µg/75 g in basic formula of cream soup and gave instant powder of infant cream soup with composition of folic acid 125.619 µg/mL, dissolved protein 2.93 mg/mL, total solids 94.65 %, total sugars 211.33 mg/mL, and reducing sugars 83.48 mg/mL. The optimum formulation takes place an increase of dissolved protein in instant powder of infant cream soup 25.75 %, total solids 2.89 %, total sugars 91.85 %, and reducing sugars 100.62 % (1 times) compared to control instant of infant cream soup. Instantiation process increases dissolved protein 113 % (1.13 times) compared to control. Identification of folic acid monomer of powder cream soup and instant cream soup are dominated by folic acid monomer with molecular weight (MW) 442.06 Dalton (Da.) and 442.65 Da., types of dominant volatile compound, linoleic acid (22.78 %) and palmitic acid (45.23 %), distribution of particle size with particle size 1,780.0 nm and 787.9 nm with index particle 0.975 and 0.774.

1. Introduction

A fermentation-based process is conducted on soy bean (G. soyae) (tempeh) by Rhizopus oligosporus strain C1 and broccoli (Brassica oleracea L.) or spinach (Amaranthus sp.) by Kombucha culture. To formulate nixtamalized corn from type of horse dent (Zea mays var. indentata) achieved through nixtamalized modification by lime solution (Ca(OH)2) has been performed in order to folic acid concentration as an effort to recover fortificant powder of natural folic acid [1]. Folic acid or folate (natural form) which is an essential micronutrient in the B-complex vitamins for growing and developing infant during in pregnancy, pregnant women and children has a role as precursor in nucleotide synthesis to remethylation of homocysteine in period of fission and growth of cells, particularly to produce red blood cells and prevent anaemia [2] [3], prevent its emergence of Neural...
Tube Defects (NTDs), such as spina bifida and anencephaly on infant. Folic acid (L-glutamic acid, pteroyl-L-glutamic acid, Vitamin B9, Vitamin M, Folacin) (C19H19N7O6) is consisting of a pteridine heterocyclic, a para-amino benzoate (p-ABA) and glutamic acid [4], as shown in Figure 1.

![Figure 1. Chemical structure of folic acid](image)

Powder of natural folic acid from mixtures of soy bean/mung bean tempeh, fermented broccoli/spinach and nixtamalized corn in composition of 1:1:1 is the best composition in recovering folic acid as fortificant of folic acid [5] for optimum supplement of pregnant women on emulsification with 3% gelatin at rotation speed 9.500 rpm for 30 minutes [6]. Application of natural folic acid fortificant in preparation of instant infant cream soup for complementary feeding is priority in its role as smart support food in order to grow and develop infant and child of five years. Infant cream soup is a instant powder product produced subsequently through mixing between fortificant and base cream soup powder, homogenizing and drying. In its serving is conducted through pouring by hot water (± 80 °C) and agitating at certain ratio. Infant instant cream soup as complementary feeding for aged ≥ one (1) year is purposed as food in order to adapt infant digestive system. Formulation is carried out at the cream soup powder-to-hot water (± 80 °C) ratio in 1 part (75 g) and 10 parts (750 g) [1]. This formulation is enabled to effect on particle size distribution in infant instant cream soup which has role in adsorption of infant digestive system.

Nano particles-sized infant cream soup particles enable to adsorb folic acid and the whole components easier in digestive system of infant. In principle, use of Particle Size Analyzer (PSA) in analysis of particle distribution by means of Coulter SZ 100 (Horiba NanoPartica) is by radiating laser (λ 750 nm) through filter spatial and projection lens to form beam collector followed by penetrating through cell samples, in which suspended particles in liquid disperse light to touch it in certain characteristic according to particle size. Distribution of particle size is calculated according to Fraunhofer method or Mie as a result of measurements 126 detectors placed with angle of 35o from optical axis [7; 8]. On identification of folic acid monomer through Liquid Chromatography coupled with Mass Spectrometry (LC-MS) is performed to know domination of its monomer based on relative specific intensity in each monomer indicating characteristic of cream soup. LC-MS will separate molecular mixture according to difference in migration speed and distribution of molecule in stationary phase (adsorbent) and mobile phase (eluen), meanwhile MS will ionize analit based on principle of electro spray ionization (ESI) to gas phase (fine aerosol) [9]. Use of Gas Chromatography-Mass Spectrometry (GC-MS) will separate different compounds of cream soup based on its volatile level by flowing mobile phase (gas) and brings stationary phase (biscuit extract) in column. Further, compound spectra are collected out from column of Mass Spectrometry (MS) [10].

The experiment activity aims to find out optimization condition on type and folic acid concentration in the best fortificant for preparing instant powder of infant cream soup as complementary feeding for aged ≥ one (1) year, its application as pour cream soup on the best composition, characteristic on folic acid monomer, type of volatile compounds, size and distribution of particles.
2. Materials and Methods

2.1. Materials and Equipment
Main materials used in this experimental activity were perforated plastic, distilled water, mung beans, broccoli and spinach purchased from a local market, dry yellow and white corn from type of horse dent (Zea mays var. indentata) purchased from a local corn plantation center (South Tangerang), sucrose (local), inoculum of Rhizopusoligosporus strain C1 and Kampuchea culture (Research Center for Chemistry – LIPI), wheat flour (local, Bogasari), corn starch (Honig, Netherland), salt, powder full milk, dry carrot (local), dry Bombay onion, dry onion leaves, white pepper powder, garlic powder. Meanwhile, chemicals used in these process and analysis were Ca(OH)2 (E. Merck), standard folic acid (Aldrich), hydrochloric acid (E. Merck), sodium nitrite (E. Merck), and methanol (E. Merck). All the chemicals used in this process and analysis were of reagent grade quality, procured locally, and used without further purification.

Main equipment utilized in this experimental activity were balance (Fujitsu, Japan), autoclave (CHENG YI, LS – 50 L, China), a series of microbiology process equipment, system of laminar flow chamber (local), incubator (local), a series of system of nixtamalization process in laboratory scale, homogenizer (Ultra-Turrax, IkaLabortecnik, T50, Jane & Kunkel, Germany), cabinet dryer, blender (local, National), sieve of 80 and 100 mesh (Retsch, Germany), and container. Main instruments for analysis were UV-vis Spectrophotometer (Model RF-550, Shimadzu, Japan), Liquid Chromatography-tandem Mass Spectrometry (LC-MS) (Mariner Bio spectrometry) with LC (Hitachi L 6200), Particle Size Analyzer (PSA) (Horiba NanoPartica-Backman) and Gas Chromatography-Mass Spectrometry (GC-MS) (Agilent Type 5977A Series, USA).

2.2. Experimental Design
The experiment work was carried out by using fortificant powders A (mixtures of nixtamalized yellow corn, mung bean tempeh and fermented spinach), B (mixtures of nixtamalized yellow corn, mung bean tempeh and fermented broccoli), C (mixtures of nixtamalized white corn, mung bean tempeh and fermented spinach), and D (mixtures of nixtamalized white corn, mung bean tempeh and fermented broccoli) added to formula soup base as folic acid at concentrations 0, 100, 200, 300, 400, and 500 µg/75 g basic formula), homogenized and dried. Analysis was performed on total solids (Gravimetric method), dissolved protein [11], total sugars (Phenol Sulphate method), reducing sugars (Somogyi Nelson) [12], and folic acid (LC-MS method). Identification on folic acid, identification on volatile compounds, and distribution of particle size were conducted by means of LC-MS (Mariner Bio spectrometry) with LC (Hitachi L 6200) [13], GC-MS instrument [10], Particle Size Analyzer (PSA) [7]. All experiment data obtained from the chemical analysis was conducted in mean values on at least triplicate analyses and evaluated descriptively.

2.3. Procedure

2.3.1. Fermentation process of mung beans (tempeh)
A number of mung beans were subsequently sorted, washed, blanched for 30 – 45 minutes, cooled, and steeped at pH 5 overnight. Next day, processed beans were hulled, washed, allowed to warm, inoculated with tempeh inoculum of Rhizopus oligosporous strain-C1 at concentration 0.2 % (w/w) uniformly, packaged in perforated plastic, and incubated at room temperature (28 – 30 °C) for 24 – 36 hours [1].

2.3.2. Fermentation process of broccoli and spinach
Broccoli and spinach was blanched at 80 °C for 5 minutes and 15 minutes, respectively, and pulverized at the vegetables-to-water ratio in 1 : 4 to produce vegetable suspension as substrate for get folic acid. Vegetable suspension was then pored by appropriate vegetable inoculum with types of vegetables of 15 % (v/w, vegetable suspension) and sucrose 10 % (w/w, vegetable suspension), and
stored in closed container with cheese cloth in dark room and room temperature for 3 days and 6 days, respectively. All activities were conducted aseptically. Biomass produced was fermented vegetable suspension [1].

2.3.3. Nixtamalization process
Some of yellow corn of horse dent and white corn of horse dent was subsequently washed and steeped in water at the ratio of corn and water of 1:4. On both steeped yellow corn was then added Ca(OH)2 of 20 % (w/w dissolved corn), cooked at 90 °C for 60 minutes, cooled, rinsed to remove cooking water and excess lime, allowed, grounded and sieved through 80 mesh to produce nixtamalized corn powder composed of different kinds of particles [14].

2.3.4. Mixing, homogenizing, drying, size reduction and sieving
Preparation of instant powder of infant cream soup was performed based on percent daily values (PDV) on folic acid according to Standard Nasional Indonesia (15) for infant food (± 400 µg/day) so that it is predicted that 1 (one) serving contains ± 75 – 150 µg with weight 75 g cream soup powder and adding water 750 mL as intake for 1 day. Base formula of infant cream soup consist of wheat flour (26.7 %), maizena (53.3 %), full cream milk (5 %), salt (5 %), sucrose (2 %), dry carrots (3.4 %), dry Bombay onion (2.4 %), dry onion leaves (0.5 %), white pepper powder (0.2 %), and garlic powder (1.5 %). Mixing process was conducted by addition of natural folic acid calculated as fortificants 0, 200, 400, 600, and 800 µg/75 g in basic formula of infant cream soup. The whole processes schema is showed in Figure 2.

![Diagram preparing instant powder of infant cream soup complementary feeding and its application by using fortificant powder of folic acid from mixture of nixtamalized corn, mung bean tempeh and fermented vegetables (broccoli/spinach)](image)

**Figure 2.** Diagram in preparing instant powder of infant cream soup as complementary feeding and its application by using fortificant powder of folic acid from mixture of nixtamalized corn, mung bean tempeh and fermented vegetables (broccoli/spinach)
3. Results and Discussion

3.1. Characteristics of materials

Infant instant cream soup as complementary feeding is produced by using fortificant from a mixture of mung bean tempeh, fermented vegetables, and nixtamalized corn as source of natural folic acid. Fortificant composition is related to composition from each material and applied processes. From composition of each material, the highest dissolved protein (3.04 mg/mL) was achieved from mung bean tempeh higher compared to nixtamalized white corn (0.22 mg/mL), nixtamalized yellow corn (0.18 mg/mL), and fermented broccoli (0.57 mg/mL) and fermented spinach (0.71 mg/mL). Fermentation process by enzymatic activity of protease of Rizosphorusoligosporus strain C1 convert mung beans to amino acids, particularly glutamic acid as component to form folic acid as dissolved protein [1]. As source of carbohydrate, both nixtamalized yellow and white corn [16] result the highest total sugars 453 mg/mL and 506 mg/mL compared to other materials. Nixtamalized yellow corn yield also the highest total solids (54.46 %) which has role on the whole compositions and fortificant texture. On reducing sugars, fermentation process of broccoli/spinach by kombucha culture produces the highest reducing sugars 61.63 mg/mL and 21.31 mg/mL, and higher compared to soy bean tempeh and nixtamalized corn. This fermentation uses sucrose as carbon source to generate folic acid through synthesis de novo [17] in which Kombucha culture as microbes convert components in vegetable to simpler and smaller components, particularly polyphenol. Remained reducing sugars from this synthesis is dissolved in biomass and is source of energy affecting on taste of application products. All raw materials used in preparation of fortificant of natural folic acid, as seen in Figures. 3a, 3b, 3c, 3d, and 3e.

![Figure 3](image-url)  
**Figure 3.** (a) Nixtamalized white corn, (b) nixtamalized yellow corn, (c) mung bean tempeh, (d) fermented broccoli and (e) fermented spinach.

Process of formulating, homogenizing, drying, size reduction, and sieving on mixture powder of fermented broccoli/spinach (1 part), mung bean tempeh (1 part), and nixtamalized yellow corn/white corn (1 part) produces fortificants with different composition on four types of fortificant powder. Formulation combination is fortificant powder A (mixtures of nixtamalized yellow corn, mung bean tempeh and fermented spinach); B (mixture of nixtamalized yellow corn, mung bean tempeh and fermented broccoli); C (mixture of nixtamalized white corn, mung bean tempeh and fermented spinach); and D (mixture of nixtamalized white corn, mung bean tempeh and fermented broccoli). Their compositions show difference, particularly on dissolved protein and total solids. This matter is influenced by compositions of initial material, ratio, and drying process so that it effects on texture, color, and particle size in fortificant powder. The whole compositions of material and fortificant are displayed in Table 1, meanwhile Figures 4a, 4b, 4c, 4d, and 4e demonstrate subsequently types of fortificant A, B, C, D, and basic formula of infant instant cream soup.

![Figure 4](image-url)  
**Figure 4.** (a) Fortificant A, (b) Fortificant B, (c) Fortificant C, (d) Fortificant D and (e) base formula of soup.
### Table 1. Material compositions in formulation of infant cream soup for complementary feeding

| Kind of material* | Component | Dissolved protein (mg/mL) | Reducing sugars (mg/mL) | Total sugars (mg/mL) | Total solids (%) |
|-------------------|-----------|--------------------------|-------------------------|---------------------|-----------------|
| Nixtamalized white corn | 0.22 | 5.24 | 4.53 | 50.47 |
| Nixtamalized yellow corn | 0.18 | 1.27 | 5.06 | 54.01 |
| Mung bean tempeh | 3.04 | 25.72 | 46.42 | 27.59 |
| Fermented broccoli | 0.57 | 61.63 | 65.03 | 8.09 |
| Fermented spinach | 0.71 | 21.31 | 49.77 | 7.66 |
| Fortificant A* | 8.83 | 77.67 | 41.16 | 87.50 |
| Fortificant B* | 6.46 | 47.55 | 81.65 | 89.86 |
| Fortificant C* | 7.44 | 17.62 | 94.28 | 93.13 |
| Fortificant D* | 5.07 | 13.16 | 74.05 | 89.68 |

Legend:*Fortificant powder A (mixture of nixtamalized yellow corn, mung bean tempeh and fermented spinach); B (mixture of nixtamalized yellow corn, mung bean tempeh and fermented broccoli); C (mixture of nixtamalized white corn, mung bean tempeh and fermented spinach); and D (mixture of nixtamalized white corn, mung bean tempeh and fermented broccoli).

### 3.2. Effect of formulation on composition of infant cream soup powder

#### 3.2.1. Dissolved protein (mg/mL) and total solids (%)

Preparation process of infant instant cream soup powder through materials mixing steps are subsequently mixing powder fortificant of nixtamalized corn (yellow or white), mung bean tempeh and fermented vegetables (spinach or broccoli) with basic formula of cream soup, homogenizing at 8000 rpm for 30 minutes, and drying by means of cabinet dryer at 50 °C for 24 – 36 hours. Adding folic acid becoming more and more high produces fluctuated dissolved protein in cream soup powder until it is yielded the highest optimization of dissolved protein. Increasing dissolved protein is caused by contribution of fortificant on basic formula cream soup, meanwhile decreasing dissolved protein is caused by its occurrence of protein denaturation during drying process. This matter causes its occurrence of lysis as a result of caramelization and formation of brown pigments of melanin or a part volatile compounds so that it is not detected as dissolved protein according to Lowry method [12].

Fortificant C generates dissolved protein in the highest cream soup with folic acid concentration 600 µg (2.93 mg/mL) compared to fortificant A (2.65 mg/mL), fortificant B (2.51 mg/mL), fortificant D (2.40 mg/mL) with folic acid concentrations of 800, 800, and 600 µg/mL, respectively, as showed in Figure 5a. This matter indicated that interaction between type of fortificant and process (mixing, homogenizing and drying) effect on dissolved protein. Difference in optimization of dissolved protein is caused by interaction between each component during homogenizing and drying and difference in initial material composition prior to process. Dissolved protein in fortificant A (8.83 mg/mL) and fortificant C (7.44 mg/mL) were higher than those fortificant B (6.46 mg/mL) and fortificant D (5.07 mg/mL) so that interaction between processes of mixing, homogenizing and drying, and basic formula of cream soup generate specific composition on each type of cream soup powder. Mung bean tempeh gives the best contribution of dissolved protein (3.04 mg/mL) in fortificant compared to both nixtamalized corn and fermented vegetables. In this optimum condition, fortificant C increases higher dissolved protein (25.75%) in cream soup powder compared to fortificants A (13.73%), B (7.72%) and D (3.0%), and cream soup powder without fortificant (0%) 2.33 mg/mL. Adding folic acid in fortificant with concentration becoming more and more increase gives fluctuated total solids in cream soup on four (4) types of cream soups, as demonstrated in Figure 5b. Optimization of higher total solids is achieved by adding fortificant B with folic acid concentration 800 µg/base formula of cream soup (94.78%) compared to fortificant A (94.25%), fortificant C (94.65%) and fortificant D (94.65%). In this condition, cream soup with fortificant B increases higher total solids (3%) compared to cream soups with fortificants A (2.5%), C (2.89%) and D (2.89%), and cream soup without fortificant (0%) 91.99%. This increase of total solids not only is caused by contribution of fortificant on basic formula of cream soup, but also by drying causing evaporation of water mass. Adding fortificant becoming more and more increase produces cream soup with the optimum total solids to a boundary, in which higher fortificant concentration will lysis or dissociate components as a result of caramelization and
forming brown pigments of melanoid in or partial dissociation as volatile compound so that it is not detected as total solids according to Gravimetric method [12].

Figure 5. Relationship between types of fortificants and folic acid concentration in fortificant on recovery of (a) dissolved protein and (b) total solids in infant instant cream soup as complementary feeding

3.2.2. Total sugars (mg/mL) and reducing sugars (mg/mL)

Total sugars are the whole carbohydrate components in various infant instant cream soup and dominated by nixtamalized corn from fortificant, wheat flour, and corn starch on basic formula to yield cream soup as source of energy, as well. Formulation of cream soup using four (4) types of fortificants with folic acid concentration in fortificant becoming more and more increase generates increasing total sugars of cream soup in the whole treatments and the best treatment is achieved in folic acid concentration 800 µg/75 g in basic formula of cream soup, as indicated in Figure 6a. Infant instant cream soup with fortificant A, B, C, and D gave the optimum total sugars in adding folic acid equivalent to 800 µg/75 g in basic formula of cream soup 192.33, 199.15, 211.33, 194.20 mg/mL, respectively or the optimum using fortificant C. In this optimum condition, adding fortificant C increases total sugars 122.26% (1.22 times) and higher compared to adding fortificants A 102.28% (1.02 times), B 109.45% (1.09 times), and D 104.25% (1.04 times) compared to total sugars in cream soup without fortificant (0%) 95.08% (0.95 times). This increase is caused by contributing fortificant on basic formula of cream soup. This matter is related to total sugars in initial material, in which fortificant C has the best total sugars (94.28 mg/mL) compared to other fortificants. Dropping total sugars are enabled to be takes place by caramelization during drying due to formation of melanoid as a consequence of Maillard reaction [18], which is not detected according to Phenol Sulphate method [12].

Different trend seems at recovery of more fluctuate reducing sugars on recovery of reducing sugars on recovery of more fluctuate, in which optimization is reached by fortificant C with folic acid concentration in fortificant of 200 µg/75 g in basic formula of cream soup 87.90 mg/mL, and higher compared to fortificants A, B, and C, and the optimum reducing sugars were reached in concentration of 800, 800, and 600 µg/75 g in basic formula of cream soup 87.87, 62.45, and 76.25 mg/mL, as demonstrated in Figure 6b.

In this optimum condition, adding fortificant C increase reducing sugars 111.25 % (1.1 times) compared to reducing sugars in cream soup without adding fortificant C (0%). This increase is also higher compared to adding fortificants A 111.22 % (1.11 times), B 50.12% (0.50 times) and D 83.29 % (0.83 times), and reducing sugars in cream soup without fortificant (0%), 41.60% (0.41 times). Reducing sugar is sugar having an ability to reduce aldehyde group or free keto in chemical structure, such as glucose, fructose, lactose, maltose, galactose, etc. which becomes parameter its occurrence of cream soup formulation. Fortificant is source of reducing sugars produced from fermentation processes (in tempeh and vegetables) by enzyme activity (amylase). These sugars are easier to enter Kreb’s cycle in order to form energy compared to non-reducing sugars (sucrose and polysaccharose).
3.2.3. Optimum condition of formulation process in instant powder of infant cream soup

From evaluation on the best composition in preparation powder of infant cream soup as complementary feeding based in the highest dissolved protein as an indication in recovering the best folic acid. The optimum process condition was achieved at concentration of fortificant C equivalent to folic acid 600 µg/75 g in basic formula of cream soup. In this condition is yielded powder of infant cream soup with composition of folic acid 125.619 µg/mL, dissolved protein 2.93 mg/mL, total solids 94.65%, total sugars 211.33 mg/mL, and reducing sugars 83.48 mg/mL. In this optimum formulation, using fortificant C increase dissolved protein on powder of infant instant cream soup 25.75%, total solids 2.89%, total sugars 91.85%, and reducing sugars 100.62% (1 times) compared to infant instant cream soup without fortificant (0%) for each component.

Pouring powder of instant cream soup at the cream soup powder-to-hot water (± 80°C) ratio of 75 g and 750 mL produces cream soup suspension. Composition after pouring indicate concentrations of folic acid 275.511 µg/mL, dissolved protein 0.26 mg/mL, total solids 9.36%, total sugars 163.27 mg/mL, and reducing sugars 8.07 mg/mL. In pouring process is occurred an increase of dissolved protein 113% (1.13 times) compared to pouring cream soup without adding fortificant C (0 µg/mL/control) from 0.23 mg/mL to 0.26 mg/mL. Figures 7a and 7b shows cream soup powder with fortificant C at folic acid concentration 600 µg/mL and cream soup suspension with fortificant C at concentration 75 g/750 mL of water (1:10), respectively.

3.2.4. Identification of folic acid monomer

Identification of folic acid monomer on standard folic acid with mass spectra m/z 439 -448 showed monomer domination with molecular weight (MW) subsequently 442.32, 442.60, and 442.93 Dalton (Da.) with relative intensities 100%, 41.63%, and 27.5%, as seen in Fig 8a. It had been known that folic acid has MW 441 Da. By means of LC-MS method had been known that a compound indicated difference in MW, in which its possibility is as M+, M+ Na+, 2M++ or 2M+, Na+. This matter is
caused by its presence of ionization process as a consequence of sensitivity of LC-MS instrument related to eluent used. Operation condition of LC-MS is injection volume 5 μL, flow rate 0.2 mL/minute using eluent mixture at the methanol-to-water ratio in 80:20, using a 15 mm x 2 mm i.e., C8 column [13]. Based on the best treatment (fortificant C, folic acid 600 µg/75 g in basic formula of soup), identification of folic acid monomer on powder of infant cream soup shows mass spectra m/z 441.879 - 443.063 with six (6) monomer of folic acid dominated by monomer with MW 442.06 Da. and relative intensity 100%, as shown in Fig 8b, meanwhile pour cream soup demonstrates mass spectra m/z 441.90 - 443.30 gives seven (7) monomer of folic acid dominated by monomer with MW 442.65 Da. and relative intensity 100%, as displayed in Figure 8c. The whole monomers of folic acid in powder and infant instant pour cream soup using fortificant C is seen in Table 2.

![Figure 8](image_url)

**Figure 8.** (a) mass spectra of standard folic acid, (b) mass spectra of infant cream soup powder using fortificant C at folic acid 600 µg and (c) mass spectra of infant pour cream soup in hot water (± 80°C) by using fortificant C.

**Table 2.** Dominant of folic acid monomer from mass spectra of standard folic acid, infant cream soup infant powder and infant instant pour cream soup in fortificant C at 600 µg/75 g in basic formula of infant cream soup.

| Kind of materials | Standard folic acid | Powder of infant cream soup | Infant pour cream soup |
|-------------------|---------------------|-----------------------------|------------------------|
|                   | Index | Centroid Mass | Relative Intensity (%) | Area | Index | Centroid Mass | Relative Intensity (%) | Area | Index | Centroid Mass | Relative Intensity (%) | Area |
|                   | Index | Centroid Mass | Relative Intensity (%) | Area | Index | Centroid Mass | Relative Intensity (%) | Area | Index | Centroid Mass | Relative Intensity (%) | Area |
| 148               | 442.3291 | 100 | 298.40 | 149 | 442.6013 | 41.63 | 10.43 | 150 | 442.9292 | 27.50 | 14.57 |
| 3270             | 442.08416 | 8.80 | 5.03 | 3271 | 442.30869 | 13.20 | 16.09 | 3240 | 442.19884 | 7.97 | 6.02 |
| 3272             | 442.38503 | 8.80 | 8.05 | 3241 | 442.38657 | 7.97 | 4.18 |
| 3273             | 442.60534 | 6.60 | 6.03 | 3242 | 442.44322 | 11.95 | 2.01 |
| 3274             | 442.81740 | 4.39 | 4.02 | 3243 | 442.56294 | 23.91 | 6.52 |
| 3275             | 442.94615 | 6.59 | 4.52 | 3244 | 442.65274 | 23.91 | 6.52 |
| 3245             | 442.85879 | 7.97 | 9.03 | 3246 | 442.98208 | 15.94 | 4.01 |

3.2.5. Distribution of particle size

Infant cream soup powder is produced through formulating process covering mixing between basic formula of cream soup and fortificant powder, homogenizing and drying, whereas ready-to-serve cream soup is produced through pouring by adding hot water at the cream soup powder to hot water (± 80°C) ratio of 1 part and 10 parts. Both process treatments predicted will possibility affect on size and particle distribution. Table 3 summarizes particle distribution of cream soup powder with fortificant C yielding larger particle size (1,780.0 nm) with index particle 0.975 compared to instant cream soup with same fortificant 787.9 nm and index particle 0.774.

This difference is not only caused by difference in material type, but also by processes. In preparation process of infant cream soup powder, using homogenizer at 8000 rpm for 30 minutes causes its occurrence particle size reduction. Particle size becoming more and more small by drying will evaporate water mass. On pouring process is occurred gelatinization of cream soup powder so that it takes place swelling wheat granule (26.7%)/corn starch (53.3%) and interaction between fortificant
and other materials through homogenizing causing smaller particle size. Another possibility is cream soup powder and fortificant C have higher total solids concentration (94.65%) compared to pour cream soup (9.36%) so that particle size is larger. Total solids are accumulation of the whole components both soluble and insoluble according to Gravimetric method [12]. On dispersed index particle, according to DLS method, in which particle size becoming more and more small will be small index particle, in other words dispersion of particles will be more uniform and more homogen. Both types of infant cream soup have index particle < 1 indicating more homogen particle size, while index particle > 1 displays ununiform particle size [7]. This difference seems in Figure 9a, in which distribution of particles from infant cream soup powder with fortificant C generates particles ranging from diameter size (Ø) 300 nm to 1200 nm (< 1,000 nm) at frequency 15% and this particle size becomes large to > 1,500 nm at frequency 65%. In the whole treatments, it is yielded particles with Ø from 100 nm to 2000 nm at frequency (dispersion of particles) 65%, whereas pour cream soup with same fortificant (C) results particles with Ø from 600 nm to 6000 nm (< 10000 nm) at frequency 11% and increase to frequency > 14% as demonstrated in Figure 9b.

| Table 3. Particles characteristic of infant instant cream soup powder and infant pour cream soup using fortificant C. |
|--------------------------------------------------|
| Kind of powders fortificant | Distribution of nano-folate particles (nm) |
|-----------------------------|-------------------------------------------|
| Infant cream soup powder using Fortificant C at folic acid concentration 600 µg. | Z-Average (nm)*: 1780.0, PI**: 0.975 |
| Infant pour cream soup using Fortificant C at folic acid concentration 600 µg. | Z-Average (nm)*: 787.9, PI**: 0.774 |

Legend: *Diameter of nano particles and **dispersed particles (Index Particle).

**Figure 9.** Distribution of particles from (a) instant cream soup powder and (b) pour cream soup using fortificant Cat optimum formulation (600 µg/75g in basic formula of soup) as complementary feeding.

### 3.2.6. Identification of volatile compounds in infant cream soup powder and pour cream soup

Identification of volatile compounds are conducted on infant cream soup powder and pour cream soup in which every result of formulation uses fortificant C with concentration 600 µg/75g in basic formula and pouring result at the cream soup powder-to-hot water (± 80 °C) ratio of 1 part: 10 parts. Infant cream soup powder displays chromatogram with 12 dominant peaks as volatile compounds dominated by volatile compound at peak 13 with Retention Time (RT) 21.786 with similarity level 99% as Linoleic acid (C18H32O2, molecular weight 280.4472 Da.) of 22.777%, as shown in Figure 10a.
Figure 10. Chromatogram of volatile compound at (a) infant powder cream soup and (b) infant pour cream soup using fortificant C at concentration of fortificant 600 µg/75g in basic formula of infant cream soup.

Linoleic acid is ethyl ester from fatty acids are achieved from mung bean tempeh as a result of degradation of fat of mung bean by lipase enzyme of Rhizopus oligosporus-C1 as tempeh yeast. Besides Linoleic acid, other fatty acids which is also reached are Hexadecanoic acid, 9,12-Octadecadienoic acid, 9-Octadecenoic acid, Methyl stearate, 9,12-Octadecadienoic acid, L Oleic Acid, and Pentyllinolate with concentration ranging of 1.384% to 22.777%. Fermentation process of mung bean tempeh seems to produce fatty acids dominating cream soup powder and effects on aroma and taste of cream soup products, as showed in Figure 9a. Difference in volatile compounds seems on pour cream soup with same fortificant, in which it is produced nine (9) compounds dominated by Palmitic Acid (45.232%), as indicated in Figure 10b. Other all volatile compounds are Dodecanoic acid, Meristic acid, 9-Octadecenoic acid, and Stearic acid with concentration ranging from 4.317% to 45.232%. Volatile compounds in pour cream soup product, which less, is possibility caused by partial loss of volatile compounds by pouring process with hot water. The whole volatile compounds present in both type of cream soup is showed in Table 4.

Table 4. Dominant volatile compounds in infant instant cream soup powder and pour cream soup using fortificant C as complementary feeding.

| Type of cream soup | Peak | RT  | Type of volatile compounds          | Similarity (%) | Concentration (%) |
|-------------------|------|-----|-------------------------------------|----------------|------------------|
| Infant instant    | 5    | 19.492 | Hexadecane acid                     | 98             | 1.971%           |
| soup powder       | 6    | 19.858 | n-Hexadecane acid                   | 99             | 2.703%           |
| fortificant powder of C. | 7    | 20.173 | Hexadecane acid,                   | 99             | 6.461%           |
|                   | 8    | 21.143 | 9,12-Octadecadienoic acid           | 99             | 5.118%           |
|                   | 9    | 21.194 | 9-Octadecenoic acid                 | 99             | 3.843%           |
|                   | 10   | 21.433 | Methyl stearate                     | 99             | 0.572%           |
|                   | 11   | 21.572 | 9,12-Octadecadienoic acid           | 99             | 8.315%           |
|                   | 12   | 21.622 | L Oleic Acid                        | 99             | 5.423%           |
|                   | 13   | 21.786 | Linoleic acid                       | 99             | 22.777%          |
|                   | 14   | 21.836 | Ethyl Oleate                        | 99             | 11.899%          |
|                   | 15   | 22.038 | Octatonic acid                      | 99             | 2.486%           |
|                   | 21   | 23.941 | Pentyllinolate                      | 99             | 1.384%           |
| Infant instant    | 1    | 16.031 | Dodecanoic acid                     | 98             | 4.688%           |
| pour cream soup   | 2    | 18.123 | Meristic acid                       | 99             | 13.970%          |
| using fortificant powder of C. | 3    | 20.056 | Palmitic Acid                       | 99             | 45.232%          |
|                   | 4    | 21.619 | 9-Octadecenoic acid                 | 99             | 24.137%          |
|                   | 5    | 21.715 | 13-Octadecenoic acid                | 99             | 4.317%           |
|                   | 6    | 21.840 | Stearic acid                        | 99             | 7.657%           |

4. Conclusion

Adding folic acid in fortificant with concentration becoming more and more increase on basic formula of infant instant cream soup will increase all compositions of components, particularly dissolved protein. Based on dissolved protein, optimization of fortification was achieved at type of powder mixture of nixtamalized white corn (Zea mays var. indentata), mung bean tempeh (Phaseolus
radiatus) and fermented spinach (Amaranthus sp.) stated as fortificant C with folic acid concentration 600 µg/mL and gave instant powder of infant cream soup with concentrations of folic acid 125.619 µg/mL, dissolved protein 2.93 mg/mL, total solids 94.65 %, total sugars 211.33 mg/mL, and reducing sugars 83.48 mg/mL. In this optimum formulation, using fortificant C increases dissolved protein on instant powder of infant cream soup 25.75 %, total solids 2.89 %, total sugars 91.85 %, and reducing sugars 100.62 % (1 times) compared to infant instant cream soup without fortificant (0 %) on each component. In process of instating, at the cream soup powder-to-hot water (± 80 °C) ratio in 1:10 is occurred an increase dissolved protein 113 % (1.13 times) compared to instant cream soup without adding fortificant C (0 µg/mL/control). Identification of folic acid monomer at cream soup powder and instant cream soup with fortificant C shows domination of folic acid monomer with molecular weight (MW) 442.06 Da. and 442.65 Da., volatile compounds dominated by linoleic acid (22.78 %) and palmitic acid (45.23 %), respectively, and distribution of particle in cream soup powder and instant cream soup displays particle size 1780.0 and 787.9 nm with index particle 0.975 and 0.774, respectively.

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