Modified cassava flour (mocaf) as food additive on infant instant cream soup using fortificant of natural folic acid

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Abstract. Modified cassava flour (Mocaf) can be used for food additive on the formulation of infant instant cream soup as thickener and binder of folic acid. The main objective of this study was to determine the optimum formulation condition of added mocaf and folic acid fortificant on infant cream soup for complementary feeding. Infant cream soup was prepared by adding mocaf at concentration 0, 6, 12, 18, 24, and 30% (w/w, base formula of cream soup), fortificant A (mixture of soy tempeh, nixtamalized corn, and fermented broccoli), and fortificant B (mixture of mung beans, nixtamalized corn and fermented broccoli). The optimum condition was achieved by adding mocaf at the concentration 30% and fortificant A, with composition of folic acid 191.14 μg/mL, dissolved protein 39.72 mg/mL, total solids 91.43 %, total sugars 926.73 mg/mL and reducing sugars 52.1 mg/mL. This formulation was able to increase folic acid by 10.27-folds in comparison with base formula of infant soup. LC-MS analysis showed folic acid monomer with molecular weight 442.75 m/z. Volatile compounds were dominated by acetic acid, furan and, fatty acid. Particle size distribution at quantities 10, 50 and 90% (v/w) had particle size of 10.58, 22.46, and 470.52 μm, respectively or average 141.26 μm.

1. Introduction
Instant cream soup is one of the Complementary Feedings (CF) apart from porridge and infant biscuit, which is introduced to infant with age of 6 - 24 months. Supplying infant cream soup is not only aimed for infant growth – development, but also used to adapt the digestive system since the taste, aroma, and other factors are related to the five senses of infant. Folic acid (Vitamin B9/Folacin) is one of the 13 essential vitamins required by women planning for a pregnancy, before and during early pregnancy, and the infant growth – development to prevent neural tube defects. Also, it has an important role as precursor in synthesis of nucleotide into re-methylation of homocysteine at period of fissing and growing cells, particularly to produce red blood cells and prevent the incidence of neural tube defects (NTDs) [1, 2]. Instant cream soup for infants is prepared by adding fortificant of natural folic acid in order to support the utilization of safe and natural ingredient for infants. Fortificant of natural folic acid is produced from mixture of soy (Glycine soyu) tempeh or mung bean (Phaseolus radiatus L.) fermented by Rhizopus oligosporus C1, combined with broccoli (Brassica oleraceae L.) or spinach (Amaranthus sp.) fermented by Kombucha culture, and nixtamalized yellow corn (Zea mays var. indentata) or white corn (Zea mays var. indentata) [3].

Furthermore, formulation of infant cream soup was performed by adding mocaf flour fermented by Lactic Acid Bacteria (LAB), enables to improve folic acid content in infant cream soup. Mocaf is modified cassava flour produced from fermentation with LAB microbes. These microbes induce...
characteristic alteration of cassava flour, including viscosity, gelation capability, power of rehydration, and easily dissolve. In overall, characteristic of mocaf is possibly affected by type and concentration of LAB culture, and fermentation time [4]. However, potential role of mocaf as food additive for thickener, binder, carrier, and emulsifier is less investigated.

The regulation concerning Food Additives is classified thickener or emulsifier or stabilizer agents as modified starch through acid or basic or oxidation or bleaching, and enzymatic processes as monostarch phosphate or hydroxy propyl distarch phosphate. However, it has yet specified the modified enzymatically process [5]. Mocaf possibly affects the existence of folic acid in instant cream soup, and it is closely impacted its composition and organoleptic quality. Mocaf used in the formulation of infant instant cream soup is expected to contribute in binding folic acid due to smaller size of granular and its functional group modification of cassava carbohydrate by LAB fermentation which is able to absorb and bind folic acid. Different mocaf concentration in formulation of infant instant cream soup may have different ability in binding folic acid and their physicochemical composition. Formulation of infant cream soup consist of mixture of flour and maize as source of soluble carbohydrate and other materials such as milk, dry vegetable, sugar and fortificant of folic acid from mixture of soy tempeh, fermented broccoli or spinach, and nixtamalized corn as main source of folic acid. Infant instant cream soup is formulated by equivalence to concentration of folic acid according to recommended dietary allowance (RDA) (80 µg/day or 30 – 50 µg per serving after pouring) [6].

The experimental study aims to evaluate the effect of mocaf concentration as thickener and carrier of folic acid on different type of fortificants in the formulation of infant instant cream soup and their physicochemical characteristics including folic acid, volatile compounds, and particle size.

2. Materials and Methods

2.1. Materials and equipment

Materials used in this experiment were soy beans, mung beans, broccoli obtained from a local market, dry yellow and white corn from type of horse dent procured from a local corn plantation center (South Tangerang), sucrose, inoculum of R. oligosporus strain C1 and Kombucha culture (Research Center for Chemistry – LIPI), Mocaf (Research Center for Biotechnology-LIPI), wheat flour (local, Bogasari), corn starch (Honig, Netherland), salt, powder full cream milk, dried carrot, dried onion, dried spring onion, white pepper, and garlic powder. All the chemical used in the process and analysis were analytical grade.

The equipment used in this experiment were analytical balance (Fujitsu, Japan), autoclave (CHENG YI, LS – 50 L, China), homogenizer (Ultra-Turrax, Ika Labortechnik, T50, Jane & Kunkel, Germany), blender (local, National), laminar flow chamber (local), incubator (local), cabinet dryer, and sieves of 60 and 80 mesh (Retsch, Germany). The analysis instrument used in this study were UV-Vis Spectrophotometer (Model RF-550, Shimadzu, Japan), Liquid Chromatography-tandem Mass Spectrometry (LC-MS) (Mariner Biospectrometry) with LC (Hitachi L 6200), Particle Size Analyzer (PSA) (Horiba Laser Scattering Particle Size Distribution Analyzer LA-960) and GC-MS (Shimadzu, Japan).

2.2. Experimental design

Experimental study was carried out by adding mocaf flour at concentration 0, 6, 12, 18, 24, and 30% (w/w, base formula of infant cream soup) to formula of infant cream soup consisting of base formula and fortificant A or fortificant B, equivalent to folic acid 1,000 µg, respectively. Fortificant A and fortificant B are mixture of soy tempeh, nixtamalized yellow corn and fermented broccoli, and mung beans, nixtamalized yellow corn and fermented broccoli in order to develop infant instant cream soup A and infant instant cream soup B, respectively. Analyses were carried out on initial fortificant materials and infant instant cream soup on dissolved protein (spectrophotometry) [7], total solids (Gravimetric method), total sugars (Phenol Sulphate method), reducing sugars (Somogyi Nelson method) [8] and folic acids (spectrophotometry) (Ruengsitagoon, & Hattanat, 2012) [9]. Identification of folic acid was conducted by LC-MS (Mariner Biospectrometry) equipped with LC (Hitachi L 6200) [10], distribution
of particles size by Particle Size Analyser (Horiba LS-PSA LA-960) [11], and analysis of volatile compound by GC-MS [12]. Experimental data was determined by description method using average yield in triplicate analyses from duplicate process.

2.3. Procedures

2.3.1. Fermentation process of soy bean, mung bean tempeh and broccoli. Soy bean or mung bean were sorted, washed, blanched for 30 – 45 minutes, cooled, and steeped at pH 5 overnight. Next day, processed beans were hulled, washed and inoculated with tempeh inoculum of *R. oligosporus* 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. Meanwhile, fermentation of broccoli was conducted by blanching broccoli at 80 °C for 5 minutes, and pulverized at the ratio of broccoli and water of 1:4 and resulted in broccoli suspension as substrate for folic acid. Broccoli suspension substrate was then poured into container, then added appropriate broccoli inoculum with broccoli concentration 15% (v/w broccoli suspension) and sucrose 10% (w/w, broccoli suspension). The suspension was stored in closed container with cheese cloth in dark room at room temperature for 3 days and then collected. All experimental processes were performed aseptically [3].

2.3.2. Nixtamalization process. A number of yellow corns were subsequently washed and steeped in water at the ratio of corn and water of 1:4. Then, Ca(OH)₂ were added with concentration of 20% (w/w dissolved protein of corn), and 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 yellow corn powder [3].

2.3.3. Preparation fortificant from mixture of tempeh paste, fermented broccoli and nixtamalized corn. Preparation process of mixture of tempeh paste and fermented broccoli was mixed at the ratio of tempeh paste and fermented broccoli of 1:1 [3], and homogenized at 8000 rpm for 30 minutes until it formed paste-like mixture. Nixtamalized corn was added into mixture of tempeh paste and fermented broccoli at the ratio of paste mixture and nixtamalized corn of 1 : 1, homogenized at 8000 rpm for 30 minutes and the mixture was obtained as fortificant paste. Next, the fortificant paste mixture was subsequently dried using cabinet dryer at 50 °C for 24 hours, and the sample size was reduced and sieved through 60 mesh until the desired powder was obtained, expressed as fortificant powder of A and B.

2.3.4. Formulation of instant infant cream soup using mocaf as binder of folic acid and thickener. The fortificant of natural folic acid being equivalent to folic acid 1,000 µg was subsequently added into base formula of infant cream soup consisting of mixture of wheat flour (26.7%), maize (53.3%), powder full cream milk (5%), salt (5%), sucrose (2%), dried carrot (3.4%), dried onion (2.4%), dried sliced spring onion (0.5%), white pepper (0.2%) and garlic powder (1.5%), and added mocaf flour at 0, 6, 12, 18, 24, and 30% (w/w, base formula of infant cream soup), mixed in dry condition, reduced size, sieved via 80 mesh, and packed. Infant instant cream soup was formulated according to recommended dietary allowance (RDA) on folic acid according to standard SNI No. 01-711.1, 2007 [13] for baby food (± 400 µg/day) so that it is predicted one (1) serving containing ± 75 – 150 µg) with weight 75 g powder cream soup and adding water 750 mL as intake for 1 (one) day.

3. Result and Discussion

3.1. Characteristic of materials

Preparation of instant cream soup by adding mocaf as carrier and thickener is related with composition of materials in its formulation. Initial composition of raw material prior to dry mixing process is shown in table 1. Based on the composition of each material, the highest folic acid (503.60 µg/mL) was obtained from soy tempeh, which is higher than mung bean tempeh (381.5 µg/mL), but had lower dissolved
protein (1.15 mg/mL) than that of mung bean (1.80 mg/mL). Fermentation process of soy tempeh involves protease enzyme activity from R. oligosporus strain C1 which causes protein degradation of soy tempeh to form amino acids, particularly glutamic acid as component in forming folic acid, in which expressed as dissolved protein [3]. As source of carbohydrate, modification of yellow corn through nixtamalization process [3] generates total sugars 146.84 mg/mL. Nixtamalized yellow corn also had the highest total solids (54.46%) which has main role on overall composition and fortificant. In addition, fermentation process of broccoli by Kombucha culture yields the highest concentration of reducing sugars (61.63 mg/mL) in comparison with soy tempeh and nixtamalized corn. This fermentation used sucrose as source of carbon to produce folic acid through synthesis de novo [14,15] by Kombucha culture. Remained reducing sugars from this synthesis are soluble in biomass and as a source of energy, and affect the taste of product.

Fortificant B has higher composition of folic acid, total solids, reducing sugars, and total sugars, but it has a lower composition of dissolved protein than fortificant A. This is possibly caused by difference in initial composition of material. Although, fortificant A used soy tempeh with higher concentration of folic acid (503.60 µg/mL) when compared with fortificant B using mung bean tempeh with folic acid 381.50 µg/mL. However, the presence of folic acid is also affected during processes such as homogenizing, drying, size reduction, and sieving. Folic acid is susceptible to mechanical treatment, light, and temperature [16]. Based on the composition of base formula of infant instant cream soup, it showed that folic acid is relatively high (109.09 µg/mL), however folic acid in mocaf is quite low (22.26 µg/mL). Table 1 summarizes material composition in formulation of infant instant cream soup, whereas figures 1a, 1b, 1c, and 1d shows subsequently fortificant A, fortificant B, base formula of infant instant cream soup and formula of mocaf flour.

**Table 1.** Composition of materials in formulating instant infant cream soup by using fortificant of natural folic acid and mocaf as carrier of folic acid.

| Type of materials                  | Components          |
|------------------------------------|---------------------|
|                                   | Folic acid (µg/mL) | Dissolved protein (mg/mL) | Reducing sugars (mg/mL) | Total sugars (mg/mL) | Total solids (%) |
| Mung bean tempeh                  | 381.50             | 1.80                      | 25.72                    | 46.42                | 27.59           |
| Soy tempeh                        | 503.60             | 1.15                      | 7.13                     | 60.00                | 44.02           |
| Fermented broccoli                | 113.26             | 1.25                      | 61.63                    | 65.03                | 8.08            |
| Nixtamalized yellow corn          | 466.81             | 0.68                      | 12.70                    | 146.84               | 54.46           |
| Fortificant A                       | 101.89             | 1.03                      | 23.77                    | 113.00               | 92.91           |
| Fortificant B                       | 130.54             | 0.83                      | 161.37                   | 318.75               | 93.07           |
| Base formula<sup>a</sup>           | 109.09             | 13.90                     | 27.18                    | 159.76               | 96.07           |
| Mocaf flour                        | 22.26              | 1.17                      | 5.50                     | 162.90               | 96.13           |

<sup>a</sup> Mixture of nixtamalized yellow corn, soy tempeh and fermented broccoli  
<sup>b</sup> Mixture of nixtamalized yellow corn, mung bean tempeh and fermented broccoli  
<sup>c</sup> Formula infant cream soup

**Figure 1.** (a) Fortificant A, (b) fortificant B, (c) base formula of instant porridge, (d) mocaf flour.
3.2. Effect of formulation on composition of infant cream soup

3.2.1. Folic acid (µg/mL) and dissolved protein (mg/mL). Preparing infant instant cream soup as source of folic acid is carried out through mixing the raw materials, fortificant A or fortificant B, base formula of cream soup and various mocaf concentration at rotation speed 4000 rpm for 15 – 30 minutes. In overall, it showed that the increase of mocaf concentration resulted in the increase of folic acid and dissolved protein in infant instant cream soup (Fig. 2). Both instant cream soup from fortificant A and fortificant B reached the highest folic acid (191.15 and 124.40 µg/mL) at mocaf concentration of 18% and 30%, respectively. These results were probably not only caused by dry mixing process, in which particle size from each formula and operation condition of dry mixing (4000 rpm, 15 minutes) affects on binding system of inter-components, but was also caused by difference in folic acid concentration of initial material. Folic acid in fortificant A is lower (101.89 µg/mL) than fortificant B (130.54 µg/mL) (Table 1). Thus, it required higher mocaf concentration to attain optimal folic acid (30%) in comparison with fortificant B (18%). In this optimum condition, the addition of mocaf with fortificants A and B in the formulation increased folic acid concentration by 1,027% (10-folds) and 129.05% (1.29-folds) when compared without adding mocaf, respectively.

![Figure 2](image.png)

**Figure 2.** Relationship of type of fortificant and mocaf concentration on recovery of folic acid (a) and dissolved protein (b) in infant instant cream soup.

Dissolved proteins were fluctuated and the optimum dissolved protein (45.38 mg/mL) were achieved at mocaf concentration of 18% by using fortificant A. This result is higher than that of fortificant B (41.55 mg/mL) at mocaf concentration of 30%, as shown in Figure 2b. The increment of dissolved protein concentration is not mainly due to the contribution of fortificant (fortificant A 5.8 and B 6.0 mg/mL), but also due to dissolved protein content in mocaf (1.17 mg/mL) (Table 1). Thus, the increasing of mocaf concentration resulted in the increase dissolved protein in infant instant cream soup, which has the binding ability in formulation. On the contrary, the reduction of dissolved protein is possibly caused by protein denaturation as a consequence of mechanical force during mixing process, in which lysis or partial dissociation as volatile compounds were occurred, that is unable to detect as dissolved protein according to Lowry method [8]. In optimum condition, the addition of mocaf by using fortificant A and fortificant B increased dissolved protein by 58.23% and 72.55%, respectively when compared without adding mocaf of 26.68 and 24.08 mg/mL, respectively.

3.2.2. Total sugar (mg/mL), reducing sugars (mg/mL) and total solids. Total sugars, reducing sugars, and total solids increased and then declined, as shown in Figure 3. Total sugar is the overall components in carbohydrate as source of energy. Optimization of formulation using both fortificants A and B is achieved at mocaf concentration of 30% (926.73 mg/mL) and 18% (623.75 mg/mL), as shown in Figure 3a. Total sugars were increased by 171% (1.7-folds) and 366.74% (3.67-folds) when compared without mocaf of 341.71 and 133.64 mg/mL, respectively. This increase is caused by mocaf concentration and composition of total sugars in initial material, although fortificant A has lower concentration of total sugars (78.28 mg/mL) in comparison with fortificant B (89.65 mg/mL) [8].
The reducing sugars presented a fluctuate trend. The cream soup with mocaf concentration of 30% and 12% had reducing sugars of 52.10 and 56.84 mg/mL, respectively, as demonstrated in Figure 3b. There was an increment of reducing sugars by 10.29% and 31% than cream soup with no added mocaf, which was 47.24 and 43.15 mg/mL, respectively. This is probably caused by mocaf contribution and interaction with other components during dry mixing process. Mocaf is soluble cassava starch as a result of fermentation by LAB. During fermentation process, carbohydrates are degraded by enzymes (invertase, amylase) of microbes to form monosaccharide. Meanwhile, the reduction of reducing sugar is possibly due to partial lysis during process [8].

![Graph of Total Sugar](image)

![Graph of Reducing Sugar](image)

![Graph of Total Solid](image)

**Figure 3.** Relationship of type of fortificant and mocaf concentration on recovery of total sugars (a), reducing sugars (b), and total solids (c) in infant instant cream soup.

The infant cream soup with both fortificants A and B at mocaf concentration of 18% and 12% had total solids of 91.64% and 91.42%, respectively, as indicated in Figure 3c. Fortificant B had lower total solids since it required smaller mocaf concentration than fortificant A (12% > 18%). In optimum condition, adding mocaf at dry mixing process increased total solids of 0.74% and 0.2% in comparison with no added mocaf, which were 90.97% and 91.24%, respectively.

Based on the highest folic acid content, the optimum condition was achieved at adding mocaf concentration of 30% using fortificant A, which resulted in folic acid of 191.14 µg/mL. This indicates the ability of mocaf to bind folic acid in the formulation.
3.3. Characteristic of volatile compounds from infant instant cream soup.

Identification of volatile compounds in infant instant cream soup is performed by GC-MS on infant soup cream with 30% mocaf concentration using fortificant A since it has the highest folic acid content. Figure 5 showed chromatogram of volatile compounds with 40 peaks dominated by ethyl/methyl ester, acetic acid of 40.805%. This compound was possibly incurred from mocaf flour fermented by Lactobacillus sp., which is able to produce acetic acid, lactic acid, and their derivative compounds (ethyl/methyl ester). Other volatile compounds, such as benzaldehyde, 3-hydroxy-4-methoxy of 16.576% were probably derived from aromatic compounds from tempeh by its formation of amino acid (lisin, arginin, prolin, fenilalamin and valin) during fermentation. Bitter taste was resulted from degradation or oxidation of trigliseride of soy fat with lime taste caused by glycoside. Glycoside is isoflavon consisting of genistin and daidzin to contribute off-flavor on tempeh. Other compounds are furan and their derivatives, like furfural, 5-Hydroxymethylfurfural, D-Glucopyranose, 4H-Pyran-4-one of 13.945%. These compounds are produced from the whole flour in formulation (rice flour, wheat flour, mocaf), sucrose as raw materials and fortificant. Chemical reaction between sugars and amino acids and its interaction with dry mixing process probably formed these compound derivatives. Fatty acid formed in infant instant cream soup, such as tetradecanoic acid, hexadecanoic acid, octadecadienoic acid, linoelaidic acid and Octadecenoic acid are existed at 5,427%, and fatty acids derivatives (methyl ester, such as Glycerol, 3-Methoxy-2,2-dimethyloxirane, Mercaptoacetone, 2-Proponol, Cyclopropyl carbinol, 3-Hexene, Cyclopropaneacboxamide, Ribofuranosyl and 1,3-Propanediol of 29.588%). These compounds were possibly developed during formulation of cream soup consisting of full cream milk and fortificant of folic acid, particularly fat from soy tempeh. These fatty acids give specific aroma of cream soup.

Figure 4. Infant instant cream soup by using fortificant powder and adding mocaf 30% (w/w, base formula of cream soup).

Figure 5. Volatile compounds in infant instant cream soup at mocaf concentration 30% (w/w, base formula of cream soup) by using fortificant A.
3.4. Identification of folic acid monomer
Folic acid has MW of 441 Da. Operation condition of LC-MS is at injection volume 5 μL, flow rate 0.2 mL/min. with eluent mixture of methanol and water at 80:20 ratio using column of C-8 (15 mm x 2 mm) [10]. Identification of standard folic acid standard by LCMS resulted in 1 peak at retention time (RT) at 3.3 minutes with relative intensity 100% and MW of 442.37 Da, as seen in Figure 6a and 6b.

Meanwhile, the chromatogram of infant soup cream at mocaf concentration of 30% using fortificant A showed one peak at retention time (RT) 1.6 minutes, dominated by 6 monomers of folic acid with MW ranged from 442.09 - 442.93 (Fig. 6c and 6d).

![Figure 6](image_url)

**Figure 6.** (a) Chromatogram and (b) mass spectra of standard folic acid, and (c) chromatogram and (d) mass spectra of infant instant cream soup at mocaf concentration 30% (w/w, base formula of cream soup).

3.5. Particle size and particle size distribution
The particle size distribution in infant instant cream soup showed the D10, D50 and D90 were 10.58 μm, 22.46 μm and 470.52 μm, respectively, with a mean diameter size 141.26 μm (Figure 7).

![Figure 7](image_url)

**Figure 7.** Particle size distribution in infant instant cream soup at mocaf concentration 30% (w/w, base formula of cream soup) by using fortificant A.
4. Conclusions
Mocaf flour has potential use as food additive, specifically as folic acid binder and thickener in preparation of infant instant cream soup using fortificant of natural folic acid. Based on folic acid recovery, the optimum condition was achieved at mocaf concentration 30% (w/w, base formula of cream soup) using fortificant A with compositions of folic acid 191.14 μg/mL, dissolved protein 39.72 mg/mL, total solids 91.43%, total sugars 926.73 mg/mL, and reducing sugars 52.1 mg/mL, respectively. The formulation with mocaf addition was able to recover folic acid by 1,027.06% (10.27-folds) than infant cream soup with no added mocaf. In addition, the LCMS analysis indicated the predominant monomer of folic acid with MW of 442.75 Da. Volatile compounds in infant cream soup were consisted of acetic acid compound and its derivatives 40.805%, sugar compounds and its derivatives 13.945%, fatty acid 5.427%, and fatty acid derivatives 29.588%. Particle size distribution in infant instant cream soup showed a mean diameter of 141.26 μm.

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