Potency of Modified Cassava Flour as Binder and Thickener in Formulation of Instant Infant Porridge using Fortificant of Natural Folic Acid

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Abstract. A dry mixing process in preparation instant and pour infant porridges fortified by natural folic acid of A and B, and added by modified cassava (mocav) flour as thickener and binder had been conducted. Fortificant of natural folic acid of A and B are a mixture of soy bean tempeh, nixtamalized yellow corn and fermented broccoli, and a mixture of mung bean tempeh, nixtamalized yellow corn, and fermented broccoli. This experiment aims to optimize by adding mocav flour and the best type of natural folic acid in preparing instant and pour infant porridges for complementary breastfeeding (CBF) and its characteristic on the whole composition, particularly folic acid, characteristic of folic acid monomer, volatile compounds, particle size, and particle size distribution on instant and pour infant porridges. The experiment were done on mocav flour with concentrations of 0, 6, 12, 18, 24, and 30% (w/w of base formula of infant porridge) with fortificant of natural folic acid of A and B, and pouring to produce instant and pour infant porridges A and B. The result of experiment showed that based on folic acid, process optimization was achieved on fortificant A at mocav concentration 18% (w/w, based formula of porridge) and increase folic acid 532.26% (5.30-folds), dissolved protein 226.62% (2.27-folds), total sugars 120.44% (1.20-fold), however drop total solids 4.61% and reducing sugars 14.94% in instant infant porridge compared to each components concentration in base formula of infant porridge. In this condition, volatile compounds of instant infant porridge and pour infant porridge dominated by acetic acid and its derivatives (44.21%), and furan and its derivatives (29.812%).

1. Introduction

Modified cassava (mocav) flour is a product of cassava flour processed by fermentation using the principles of modification of cassava cells. The growth of microbial enzymes during fermentation cause characteristic change of flour, such as increasing viscosity, gelation ability, power rehydration, and dissolves easily. The whole mocav characteristics are possibility affected by types and Lactic Acid Bacteria (LAB) culture concentration, and fermentation time [1]. In general, mocav is used as substitution material of wheat flour for food products, such as bakery, noodle, and cookie to get non-gluten food products. Today, the essential role of mocav as food additives for thickener, binder, and emulsifier are not explored yet. Bill of the Republic of Indonesia Number 18 Year 2012 concerning Food and Government Regulation Number 28/2004 on Food Safety, Quality and Nutrition Chapter II, Part Two concerning Food Additives differentiates between thickener or emulsifier or stabilizer agent as modified starch through acid/basic/oxidation/bleached modification and enzymatic modification, which can act as monostarch phosphate or hydroxy propyl distarch phosphate, however, it is not yet as
specific type to modify it enzymatically [2]. Mocav is possibly modified starch type in enzymatic because cassava is prepared by fermentation by LAB.

A series of formulation process is performed in preparing infant porridge as complementary breastfeeding (CBF) by adding fortificant of natural folic acid from mixture of soy beans (Glycine soya) tempeh and mung beans (Phaseolus radiatus L.) fermented by Rhizopus oligosporus C1, fermented broccoli (Brassica oleracea L.) by kombucha culture, and yellow dent corn (Zea mays indentata) by nixtamalized by Ca(OH)₂ solution [3]. Adding mocav to this formulation is not only conducted as a thickener in order to produce safer infant porridge products, but also mocav has function as a carrier of folic acid. Mocav effects on the existence of the folic acid in products as instant porridge after pour porridge and has an important essential role in composition and inorganic quality.

Folic acid (also known as folate, vitamin B9 or folacin, pteroyl-L-glutamic acid, pteroyl-L-glutamate, pteroylmonoglutamic acid, C19H19N7O6) [3] having the similar basic molecular structure consisting of 3-parts structure, pteridine ring, p-aminobenzoic acid (p-ABA), and glutamic acid molecule [4], sensitive on oxidative degradation, light, mechanical treatment and high temperature [5]. Adding folic acid is generally performed through fortification of folic acid as a result of synthesis by mixing into instant porridge flour, using natural folic acid as a result of a process aiming to explore the potency of natural resource (legumes, vegetables, cereals), and outcomeing as nutritional sources [6]. Folic acid is an essential component used to the growth and development of infants in the periconceptual period and during pregnancy. Folic acid has essential role as precursor in synthesis of nucleotide to remethylation homocysteine on the growth in cell division that is important for producing red blood cell [7;8] and prevent its emerging Neural tube defects (NTDs), such as spina bifida and anencephaly on infants. Application of fortificant of natural folic acid in preparing instant infant porridge for CBF has become a priority in its essential role as a functional food to reach smartness level. Infant porridge is food with semi solid shape with a composition according to staple food being proposed to adapt a digestive system of baby with various categories, such as instant porridge powder, ready-to-eat porridge with a result of the pour, and ready-to-cook porridge [9]. Infant porridge powder is diluted with equivalent to folic acid content according to RDA, 80 µg/day or 30 – 50 µg per serve [10]. Formulation of instant infant porridge powder and ready-to-consume it through fortificant of natural folic acid by adding mocav at various concentrations is enabled to affect on composition, particularly folic acid, particle size, and domination of folic acid monomer.

The experiment activity aims to find out effect of the best concentration of mocav as thickener and carrier of folic acid at different type of fortificant in formulating instant infant porridge powder and ready-to-consume pour porridge on the whole compositions, particularly the best folic acid and it’s characteristic on the present of the folic acid monomer, type of volatile compounds, particle size, and particle size distribution.

2. Materials and Methods

2.1. Materials and Equipments
Main materials utilised for this study were [3] distilled water, soy beans, mung beans, broccoli and spinach (local), yellow dent corn (Zea mays var. indentata) and white dent corn (local, corn plantation center, South Tangerang), sucrose (local), inoculum of Rhizopus oligosporus strain C1 and Kombucha culture (Research Center for Chemistry – LIPI), wheat flour (local, Bogasari), corn starch (Honig, Nederland), powder milk (local), butter (local), baking powder (local), salt and vanilla (local), and perforated plastic (local). Whereas, chemicals utilised in this study were Ca(OH)₂ solution (E.Merck), standard folic acid (Aldrich), hydrochloric acid (E.Merck), sodium nitrite (E.Merck), and methanol (E.Merck). All the chemicals with reagent grade used in this study were procured through local market [1]. Important equipment utilised in this study were homogenizer (Ultra-Turrax, Ika Labortechnik, T50, Jane & Kunkel, Germany), balance (Fujitsu, Japan), autoclave (CHENG YI, LS – 50 L, China),, blender (local, National), flow chamber (local), incubator (local), cabinet dryer (local), and sieves of 80 and 100 mesh (Retsch, Germany). Important instruments for analysis were Liquid Chromatography-tandem
Mass Spectrometry (LC-MS) (Mariner Biospectrometry) with LC (Hitachi L 6200), Particle Size Analyzer (PSA) (Horiba Nano Partica-Backman) [2], GC-MS (Shimadzu, Japan). and UV-vis Spectrophotometer (Model RF-550, Shimadzu, Japan).

2.2. Experimental Design

This experiment activity was carried out by adding mocav with concentration 0, 6, 12, 18, 24 and 30% (w/w, based formula of infant porridge) on porridge formula consisting of base formula and fortificant A and B, in which fortificant A are mixture of soy bean tempeh, nixtamalized yellow dent corn and fermented broccoli, and fortificant B is mung beans tempeh, nixtamalized yellow corn and fermented broccoli to get instant infant porridge A and instant infant porridge B. In this similar time, for each treatment is performed at instant or pour porridge powder in warm-hot water (± 80 – 90 ºC) on a 50 g : 160 mL ratio as being done for pour porridge C and D. Analyses were performed on initial fortificant materials and instant/pour infant porridge on total sugars (Phenol Sulphate method), total solids (Gravimetric method)[11], dissolved protein (spectrophotometry) [12], folic acids (LC-MS) [13] and reducing sugars (Somogyi-Nelson method) [14]. Identification on folic acid was conducted by LC-MS (Mariner Biospectrometry) tandem with LC (Hitachi L 6200) [15], particles size distribution (Particle Size Analyser PSA) [16], and volatile compounds using GC-MS (Shimadzu, Japan) [17]. Experimental data processing were evaluated using descriptive way from a yield of average in triplicate analyses from the duplicate process.

2.3. Procedure

2.3.1. Fermentation process of soybeans, mung beans tempeh, and broccoli

A number of soybeans or mung beans are sorted, washed, boiled for 30-45 minutes, cooled, and soaked at pH 5 overnight. The following day, processed bean were peeled, washed, inoculated with Rhizopus oligosporus tempe inoculum strain-C1 at a concentration of 0.2% (w / w) uniformly, packed in perforated plastic, and incubated at room temperature (28 - 30 ºC ) for 24 - 36 hours. Meanwhile, fermentation of broccoli is conducted by blanching broccoli at 80 ºC for 5 minutes, and pulverized at the ratio of broccoli and water of 1:4 to produce broccoli suspension as a substrate for get folic acid. Broccoli suspension was then poured by appropriate broccoli inoculum with types of broccoli of 15 % (v/w broccoli suspension) and sucrose 10 % (w/w, broccoli suspension), and stored in a closed container with cheesecloth in dark room at room temperature for 3 days. All activities were conducted aseptically. Biomass produced was fermented broccoli suspension [18].

2.3.2. Nixtamalization process

A number of horse dent- yellow corn was washed and soaked in water with a ratio of corn and water 1: 4. Then soaked yellow corn, then added Ca (OH) 2) 20% (w/w dissolved corn protein), cooked at 90 ° C for 60 minutes , cooled, and rinsed to remove excess cooking water and lime, left, ground and filtered through 80 mesh to produce nixtamalized yellow corn powder [18]

2.3.3. Mixing, homogenizing, drying, size reducing and sieving fortificant

The preparation process of mixing tempe paste and fermented broccoli was done in a ratio of 1: 1 [18], then homogenized (8000 rpm, 30 minutes) to produce a mixture of soybean tempeh and broccoli fermentation. Nixtamalized corn was added into mixture of tempeh paste and fermented broccoli on a 1:1 ratio and homogenized (8000 rpm, 30 minutes) to have resulted in fortificant paste, in treatment was subsequently dried by means of cabinet dryer (50 ºC, 24 hours), size reduction, and sieved via 60 mesh so that it is yielded desired powder as fortificant powder of A and B.

2.3.4. Preparing instant/pour infant porridge using mocav as binder and thickener of folic acid

Instant porridge for baby food was prepared according to Recommended Dietary Allowances (RDA) on folic acid in accordance with SNI [10]. Folic acid intakes according to RDA or range of 400 µg per day or 100 – 150 µg per serving of porridge. On this base formula of porridge was added fortificant of folic
acid A and B equivalent to folic acid 1,000 µg/mL (w/w of based formula of porridge). The base formula of infant porridge consisted of rice flour, skim milk powder, wheat flour, sugars of 70.12, 7.08, 5.26, 17.54 % (w/w of 100 g of base formula), and mocav in various concentrations as shown in experimental design. Instanization process was subsequently carried via by mixing rice flour and flour in the same ratio (1 part) by adding water (7 parts) at gelatination temperature (± 90 °C) in order to form paste, drying at 50 °C for 24 hours, sieving via 60 mesh, adding skim milk and sugars. Pouring is carried by stirring 50 g instant porridge in 160 mL hot water (80 – 90 °C).

2.3.5. Identification and analysis of folic acid using LC-MS
Samples instant infant porridge contain fortificant A and B (known as instant infant porridge A and B), and pour infant porridge contain fortificant C and D (known as pour infant porridge C and D). Oligomer was analyzed by a LC-MS using Mariner Biospectrometry. LC system was integrated with Q-TOP MS through Electrospray Ionization (ESI) system, in which scan mode was carried out in a mass spectra ranging m/z 100 – 1200 at 140 °C. LC (Hitachi L 6200) was carried out on a 15 mm x 2 mm C18 (RP 18) Supelco column 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].

3. Results and Discussions

3.1. Characteristics of materials
Instant and pour infant porridges use fortificant from mixtures of soy tempeh or mung beans tempeh, fermented broccoli, and nixtamalized yellow dent corn as a source of folic acid. Fortificant is related to the composition of each material and a series of processes being reached. Table 1 showed Composition in preparation of fortificant of natural folic acid.

| Kind of material          | Folic acid (µg/mL) | Total solids (%) | Dissolved protein (mg/mL) | Reducing sugars (mg/mL) | Total sugars (mg/mL) |
|---------------------------|--------------------|------------------|---------------------------|-------------------------|----------------------|
| Mung bean tempeh          | 766.56             | 53.49            | 1.80                      | 22.60                   | 286.36               |
| Soy bean tempeh           | 568.52             | 44.02            | 1.15                      | 23.60                   | 160.60               |
| Fermented broccoli        | 57.98              | 8.09             | 0.98                      | 28.28                   | 316.21               |
| Nixtamalized yellow corn  | 466.81             | 54.46            | 0.30                      | 12.40                   | 316.14               |

Preparation of instant infant porridge is performed by using fortificant of soy or mung beans tempeh, fermented broccoli, and nixtamalized yellow dent corn as source of natural folic acid. From composition of each material, the highest concentration of folic acid (766.56 µg/mL) from mung beans was higher than that soy beans tempeh (568.52 µg/mL), fermented broccoli (57.98 µg/mL) and nixtamalized yellow corn (466.81 µg/mL). This matter is related with presence of dissolved protein in mung beans tempeh (1.80 mg/mL) being higher compared with soy beans tempeh (1.15 mg/mL), fermented broccoli (0.98 mg/mL) and nixtamalized yellow dent corn (0.3 mg/mL). Difference in dissolved protein reflect activities of yeast protease enzyme of R. ologosporus C1 degrading protein to smaller and simpler amino acids, such as glutamic acid with high solubility in water [19].

Glutamic acid is part of the whole structure of the folic acid molecule besides para-aminobenzoate (p-ABA) and pteridine heterocycle [20]. This fermentation increases dissolved protein and folic acid concentrations in soy and mung bean tempeh compared with before fermentation, dissolved protein and folic acid of broccoli [21]. As a source of carbohydrate, nixtamalized yellow dent corn generates the highest concentration of total sugars (316.14 mg/mL) compared with other materials. On this material has been done a nixtamalization process of corn in order to raise its folic acid [22]. Nixtamalized yellow
Dent corn produces the highest total solids concentration (54.46%), in which other materials have also essential role on the whole composition and texture of fortificant. On reducing sugars, fermentation process of broccoli by Kombucha culture generates the highest concentration of reducing sugars (18.28 mg/mL), being higher compared with soy beans or mung beans tempeh and nixtamalized yellow dent corn. This fermentation uses sucrose as a source of carbon to produce folic acid through synthesis de novo [23] by Kombucha culture converting component of vegetable, such as polyphenol. Remain reducing sugars produced from this synthesis is a soluble component in biomass, that is the source of energy and affects on the taste of application products.

3.2. Effect of formulation on fortificant characteristic
Process of material mixing, homogenizing, drying, size reduction and sieving on mixtures of soy beans or mung beans tempeh (1 part), fermented broccoli (1 part), and nixtamalized yellow corn (1 part) produce fortificant with different composition, as tabulated in Table 2.

Table 2. Composition of materials in the formulation of instant or pour porridge using mocav as binder and thickener of folic acid.

| Kind of material                  | Folic acid (µg/mL) | Total solid (%) | Dissolved protein (mg/mL) | Reducing sugar (mg/mL) | Total sugar (mg/mL) |
|----------------------------------|-------------------|----------------|---------------------------|------------------------|----------------------|
| Fortificant A*                   | 101.89            | 92.91          | 5.80                      | 28.00                  | 78.28                |
| Fortificant B*                   | 130.54            | 93.07          | 6.00                      | 19.14                  | 89.65                |
| Base formula of infant porridge**| 109.09            |                |                           |                        |                      |
| Mocav flour                      | 22.26             | 96.13          | 1.17                      | 5.50                   | 162.90               |

Legend : *fortificant powder A : mixture of soy beans tempeh, fermented broccoli and nixtamalized yellow corn; B : mixture of mung bean tempeh, fermented broccoli and nixtamalized yellow corn.

Fortificants A and B are suspension of smooth powder with color gradation from pale brown to dark brown. The whole appearances of fortificant showed effect of processes related, particularly drying process producing reaction of browning due to melanoidine brown pigment emerged by result of reaction between sugars and protein [19]. Composition of fortificant B indicates folic acid (130 µg/mL), total solids (93.07%), total sugars (89.65 mg/mL), and dissolved protein (6.0 mg/mL) being higher, however reducing sugars (19.14 mg/mL) being lower compared with fortificant A. This matter is caused by difference in type of material and interaction amongst each process. Formulating and drying decrease folic acid, dissolved protein, and total sugars as consequence of denaturation, high temperature, however they increase total solids caused by evaporation of water mass.

Mocav flour functioning as food additive for carrying folic acid and thickening porridge have lower concentration of folic acid (22.26 µg/mL) compared with base formula of porridge (109.09 µg/mL). This matter is caused by flour formula which is combination of rice flour, wheat flour, skim milk powder and sugars so that it contributes possibility sufficient significant on the whole concentrations of folic acid. Base formula of porridge has also the highest concentration of total sugars (159.76 mg/mL) compared with other components showing main source of porridge energy, meanwhile reducing sugars indicates sugars having ability to reduce sugars to fructose and glucose contributing on taste of porridge. Figures 1a to 1h showed subsequently soy beans tempeh, mung beans tempeh, nixtamalized yellow corn, fermented broccoli, fortificant A, fortificant B, base formula of porridge flour and mocav flour, fortificant A, fortificant B, base formula of instant porridge, and mocav flour.
Figure 1. (a) Soy beans tempeh, (b) mung beans tempeh, (c) fermented broccoli, (d) nixtamalized yellow corn, (e) fortificant A, (f) fortificant B, (g) base formula of instant porridge, and (h) mocav flour.

3.3. Effect of adding mocav flour on the composition of instant and pour infant porridge

3.3.1. Folic acid (µg/mL)

Adding mocav flour on the formula of instant infant porridge by using fortificant A and B produce instant infant porridge, as shown in Figure 2a. Mocav flour concentration becoming more and more high increases folic acid in instant infant porridge to the best binding tolerance, as shown in Figure 2b.

Figure 2. Relationship between mocav concentration and type of fortificant on the recovery of folic acid in (a) instant infant porridge and (b) pour infant porridge.

Optimization is achieved on instant porridge with fortificant A at mocav concentration of 18% and gives folic acid 689.73 µg/mL. On this optimum concentration of mocav, formulation system is able to increase folic acid 532.26% (5.3-folds) compared with folic acid in the base formula of instant infant porridge (109.09 µg/mL). Increasing folic acid is possibly caused by mocav flour, which is able to absorb folic acid through the dry mixing process (6000 rpm for 5 – 10 minutes). On higher concentration of mocav is enabled to be occurred denaturation of folic acid by dry mixing system. Reaction rate of pouring on folic acid of pour porridge is demonstrated in Figure 3b, in which optimization of folic acid (542.27 µg/mL) is reached at fortificant B with mocav concentration of 12% being higher compared with folic acid of porridge (526.05 µg/mL) in fortificant A at the optimum concentration of mocav (24%). Increasing folic acid content in pour porridge is not only caused by the factor of fortificant type, but also by its occurrence of gelatinization causing swelling flour granular so that it binds more much.
folic acid. Dropping folic acid is also caused by a lower concentration of material (50 g instant porridge/160 mL) or instant porridge and hot water ratio of 1 and 3. In the optimum condition of instant porridge (fortificant A with mocav concentration of 18%), pouring process at 50 g instant powder/160 mL hot water decreases folic acid of pour porridge 35.37%.

3.3.2. Dissolved protein (mg/mL)

The term dissolved protein refers to the presence of folic acid by 3-part structure containing \( p\)-aminobenzoic acid (\( p\)-ABA) molecule linked at one end to a pteridine ring and at the other end to one glutamic acid molecule [20]. Adding mocav flour with concentration becoming more and more increase seems to increase the dissolved protein of instant porridge. Optimization is reached on instant porridge with fortificant A (4.54 mg/mL) at mocav concentration of 18% being higher compared with optimization by fortificant B at mocav concentration of 30% (4.16 mg/mL), as shown in Figure 3a. The difference in dissolved protein is not only caused by interaction among fortificant, base formula of porridge and mocav, but also by unstable protein property because of high solubility in water and treatment of dry mixing so that it is the occurrence of lysis which is not detected by Lowry method [12]. On the optimum mocav concentration (18%), formulation system using fortificant A is able to increase dissolved protein of instant porridge 226.62% (2.26-folds) compared to dissolved protein in base formula of instant infant prior to process of mixing (1.39 mg/mL).

![Figure 3](image.png)

**Figure 3.** Relationship between mocav concentration and type of fortificant on recovery of dissolved protein in (a) instant infant porridge and (b) pour infant porridge.

Trend of reaction rate on binding dissolved protein seems on pouring process of instant porridge, as displayed in Figure 3b. This matter is indicated by optimizing mocav concentration of 18% in pour porridge with fortificant A (0.66 mg/mL) being higher compared with fortificant B at mocav concentration of 30% (0.59 mg/mL). Increase of dissolved protein is not only caused by the factor of fortificant type, but also by dissolving amino acids, whereas decline of dissolved protein is caused by denaturation in using hot water (± 90 ºC). In the optimum dissolved protein of instant porridge (fortificant A with mocav concentration of 18%), pouring process at 50 g instant porridge/160 mL hot water drops dissolved protein of pour porridge 587% (5.9-folds) from 4.54 mg/mL to 0.66 mg/mL.

3.3.3. Reducing sugar (mg/mL)

A reducing sugar is any sugar that is capable of acting as a reducing agent because it has a free aldehyde group or a free ketone group. All monosaccharides are reducing sugars, such as glucose, fructose and galactose. They act as a source of energy, which eases to be absorbed and affect directly on taste of infant porridge. Adding mocav flour (30%) to instant porridge with fortificant B shows optimization of reducing sugars being higher compared with instant infant porridge with fortificant A at mocav concentration of 12% (45.42 mg/mL), as demonstrated in Figure 4a. On this optimum condition, formulation system is able to increase reducing sugars of instant porridge of 87.16% compared with reducing sugars in base formula of instant infant porridge before mixing process (27.18 mg/mL).
Increase of reducing sugars is caused by contribution of mocav, meanwhile decrease of reducing sugars is possibility caused by dry mixing system or interaction amongst other components to form compounds being undetected according to Somogyi Nelson method [13]. On pouring process of instant porridge, increase of mocav concentration in pour porridge shows similar trend, in which reducing sugars with fortificant B shows the highest mocav concentration 30% (41.60 mg/mL), whereas pour porridge with fortificant A reaches optimum condition at mocav concentration 18% (27.82 mg/mL), as shown in Figure 4b. On this optimum condition, reducing sugars in instant porridge (fortificant B, mocav concentration 30%) followed by pouring process at 50 g instant porridge/160 mL hot water decreases reducing sugars of pour porridge 18.25% from 50.89 mg/mL to 41.60 mg/mL.

Figure 4. Relationship between mocav concentration and type of fortificant on recovery of reducing sugars in (a) instant infant porridge and (b) pour infant porridge.

3.3.4. Total Sugars (mg/mL)

Total sugars acts as the whole references for carbohydrates as source of energy. Increase of total sugars concentration in instant infant porridge fluctuates, however increases on both types of fortificants. Instant infant porridge with fortificant B achieves optimization condition (420.29 mg/mL) at mocav concentration 30% being higher compared with optimization with fortificant A at mocav concentration 24% (418.32 mg/mL), as showed in Figure 5a.

Figure 5. Relationship between mocav concentration and type of fortificant on recovery of total sugars in (a) instant infant porridge and (b) pour infant porridge.

In this condition, formulation system is able to increase total sugars (420.29 mg/mL) of instant porridge of 163.53% (1.63-folds) compared with total sugars of base formula of instant porridge before mixing process (159.76 mg/mL). Difference in optimization condition of total sugars at both types of fortificant are possibility caused by difference in composition of formulation, particularly on fortificant, in which total sugars in fortificant B being higher (89.65 mg/mL) compared with fortificant A (78.28 mg/mL). It had been known that base formula of infant porridge consist of rice flour, wheat flour, skim...
milk powder, and sugars, or in other words almost all compositions are carbohydrate (> 94.74% of the whole formulas). On pouring process shows optimization of total sugars (249.45 mg/mL) is reached by pour porridge with fortificant A at mocav concentration 24% being higher compared with fortifikan B (160.25 mg/mL) at mocav concentration 30%, as indicated in Figure 5b. This matter indicates that gelatinization process of fortificant A is more efficient because it needs mocav flour with lower concentration (24%) in order to get a higher concentration of total sugars compared with gelatinization using fortificant B (30%). In this optimum condition, total sugars of instant porridge (fortificant B, mocav concentration 30%) and pouring process at 50 g instant porridge/160 mL hot water drops total sugars of pour porridge 40.84% from 420.29 mg/mL to 249.45 mg/mL.

3.3.5. Total solids (%)

Total solids is a measurement that includes the whole components both dissolved, suspended and settleable solids in water. It acts as a reference on nutrition, texture, organoleptic quality, and life time of food products. Adding mocav flour with concentration becoming more and more increase produces an optimum concentration of total solids of instant porridge with fortificant A (91.64%) at mocav concentration of 18% being higher compared with fortificant B at mocav concentration of 12% (91.42%), as displayed in Figure 6a.

![Figure 6a](image-url)

**Figure 6.** Relationship between mocav concentration and type of fortificant on recovery of total solids in (a) instant infant porridge and (b) pour infant porridge.

In this condition, the formulation system declines total solids 4.61% compared with total solids in base formula (96.07%). Declining total solids is possibility caused by interaction amongst initial fortificant, base formula, and mocav. Fortificant A has lower concentration of total solids (92.91%) than that fortificant B (93.07%), however by contributing base formula (96.07%) and mocav (18% w/w) cause higher concentration of total solids of instant porridge compared with instant porridge with fortificant B. Formulation result shows that total solids in fortificant B, base formula, and mocav at concentration 12% are 93.07%, 96.07%, and 96.13%, respectively. This matter shows that dry mixing system needs mocav with higher concentration, despite lower concentration of total solids in fortificant to get instant porridge with the optimum concentration of total solids. Similar trend of reaction rate seems to process pouring, in which optimization condition (10.10%) is reached at pour porridge with fortificant A at mocav concentration 12% being higher compared with optimization condition at fortificant B (9.84%) with mocav concentration 18%, as shown in Figure 6b. This matter showed that using mocav as thickener for fortificant A is more efficient than that fortificant B. On the optimum condition, total solids of instant porridge (fortificant A, mocav concentration 18%) and pouring process at 50 g instant porridge/160 mL boiled water drops total solids of pour porridge from 91.64% to 9.91% (80.82%).

3.4. Optimum process condition on formulation of instant and pour infant porridges

From assessment on formulation process in preparation of instant infant porridge and pour porridges by adding mocav flour as carrier of folic acid and thickener, the best formulation based recovery of folic
acid is reached by using fortificant A (mixtures of soy beans tempeh, fermented broccoli, and nixtamalized yellow corn at mocav flour concentration 18%, w/w base formula of porridge). This formula is generated instant and pour infant porridge from similar formula with composition of folic acid 689.73 and 445.76 µg/mL, dissolved protein 4.54 and 0.66 mg/mL, total solids 91.64 and 9.91%, total sugars 352.17 and 120.87 mg/mL, and reducing sugars 37.38 and 27.82 mg/mL, respectively. Figures 7a and 7b displays instant infant porridge and pour porridge using fortificant A and adding mocav flour 18% (w/w base formula of porridge).

3.5. Identification of volatile compounds in instant and pour porridge

Identification of volatile compounds is conducted on instant infant porridge and pour porridge based on the best recovery of folic acid at formulation of mocav with concentration 18% using fortificant A. Instant infant porridge demonstrates chromatogram with 19 peaks dominated by volatile compounds at peak 3 and peak 4, which are also found at peaks 7, 9, and 10 as ethyl/methyl ester acetic acid with total concentration 44.21%, as displayed in Figure 8a.

These compounds are ester, which is possibility get from mocav flour. It had been known that mocav flour is produced from fermentation process of Cassava flour by *Lactobacillus* sp., in which this process is enabled to yield acetic acid, lactic acid, and their derivative compounds (ethyl/methyl ester). Other volatile compounds are furan compounds and their derivatives, such as furfural, 5-hydroxymethylfurfural, -D-Glucopyranose, 4H-Pyrان-4-one with total 8.75%. These compounds are yielded from the whole flours in formulation (rice flour, wheat flour, mocav) and on fortificant of folic acid are calculated as carbohydrate, their fatty acid derivatives (propanoic acid, 2-propanone, propanoic acid, propylamine with total 11.81%), butanoic acid and their derivatives (2,3-Butanediol) with total 6.412%, and other methyl ester (mercaptoacetone, 3-methoxy-2,2-dimethyloxirane, thylhydroxylamine) with total 6.553%.

Pour infant porridge displays chromatogram with 28 peaks, as showed in Figure 8b dominated by volatile compounds at peak 1, 2, 12, and 17 as ethyl/methyl ester acetic acid, such as acetic acid,
fluoroacetic acid, hydrazinecarboxylic acid with concentration total 17.811%, furan compound and their derivatives furfural, 2-furanmethanol, 2(5H)-furanone, 2H-pyran, 4H-pyran-4-one, tetrahydro-4H-pyran, 5-hydroxymethylfurfural with total 29.812%, alcohol derivatives (1-butanol, ethanol, ethan, propylene glycol, 1,2,15-pentadecanetriol, 1,3-butandiol) with total 16.127%, fatty acids (n-hexadecanoic acid, 9,12-octadecadienoic acid, 9,12-octadecadienoic acid) with total 5.84%, and other volatile compounds, such as methyl ester (3-amino-2-oxazolidinone, L-1-butynie, 2-cyclopenten-1-one, 1,3dioxane, 1,3-diaminoguanidine, formic acid, 2-ethoxyethyl acetate, 1-propanamine) with total 19.96%. It had been seemed that dry mixing process is dominated by acetic acid and their derivatives, and only a less part of sugar (as furan and their derivatives), fatty acid (propanoic acid and butanoic acid), and other methyl ester, meanwhile pouring process is dominated by sugars (as furan and its derivative), the presence of alcohol compounds, less acetic acid and fatty acids. The whole dominant volatile compounds from both type of infant porridges are indicated in Table 3.

Table 3. Dominant volatile compounds from instant infant porridge and (b) pour infant porridge at optimum condition (fortificant A and mocaf flour 0.3% w/w base formula of porridge).

| Kind of porridge | Kind of compound | volatile (%) | Kind of porridge | Kind of volatile compound | (%)* |
|------------------|-------------------|-------------|------------------|--------------------------|------|
| Instant infant porridge | 2-Propanone | 2.87 | Pour infant porridge | Propanoic acid | 2.09 |
| | Silanediol | 2.72 | | L Acetic acid | 10.60 |
| | Acetic acid | 16.40 | | LFluoroacetic acid | 2.34 |
| | Acetic acid | 25.48 | | L-1-Butanol | 0.59 |
| | Propanoic acid | 5.23 | | Methyl glyoxal | 2.05 |
| | Propylamine | 0.63 | | Butyric acid hydrazide | 0.47 |
| | 2,3-Butanediol | 2.57 | | Propanoic acid | 11.86 |
| | 2,3-Butanediol | 1.87 | | L 1-Butyne | 2.10 |
| | Thylhydroxylamin | 1.86 | | L Furfural | 1.78 |
| | Acetic acid | 2.33 | | Furamnethanol | 11.85 |
| | Furfural | 4.86 | | Acetic acid | 3.19 |
| | 2-dimethyloxiran | 2.82 | | Propylene Glycol | 6.58 |
| | 2-Propanone | 3.08 | | 2(5H)-Furanone | 2.36 |
| | Mercaptoacetone | 1.87 | | Glyceraldehyde | 1.40 |
| | Butanoic acid | 1.01 | | 2-Cyclopenten | 5.05 |
| | 4H-Pyran-4-one | 0.99 | | L Acetic acid | 1.93 |
| | Hydroxy methylfurfural | 1.14 | | Pentadecanetriol | 2.64 |
| | Butanoic acid | 11.52 | | 6L 2H-Pyran | 0.997 |
| | D-Glucopyranose | 1.76 | | Methylpropyl ester | 2.537 |
| | | | | L Formic acid | 1.980 |
| | | | | 1,3-Butanediol | 1.451 |
| | | | | 1-Proponamine | 2.934 |
| | | | | 4H-Pyran-4-one | 7.072 |
| | | | | 4-Furalidol | 6.324 |
| | | | | Hydroxymethylfurfural | 6.939 |
| | | | | n-Hexadecanoic acid | 2.061 |
| | | | | Octadeadadienoic acid | 3.779 |

Legend: *Retention time; **concentration.

3.6. Identification of folic acid monomer in instant and pour infant porridges

Identification on standard folic acid is found at 1 peak (T 3.3) with retention time of 0 – 10 minutes with relative intensity 100%, in which at mass spectra m/z 439 – 448 from T 3.3 displays domination of monomer with molecular weight (MW) of 442.37 Dalton (Da.) and relative intensity 100%, as demonstrated in Figures 9a and 9b. It had been known MW of folic acid is 441 Da. By means of LC-MS instrument, it had been given that a compound has possibility of M+, M+ Na+, 2M++ or 2M+, Na+ stated as difference in MW. Operation condition of LC-MS is injection volume 5 µL, flow rate 0.2 mL/min. with eluent mixture of methanol and water at a 80 : 20 ratio using column of C-8 (15 mm x 2
Identification on folic acid monomer is performed on samples of instant infant porridge and pour porridge from the best treatment based on folic acid using treatment with fortificant A at mocav flour concentration 18%. Figures 9c and 9e demonstrate chromatogram of instant infant porridge and pour infant porridge dominated by peak T 1.5 with retention time range 0 – 10 minutes and relative intensity 100%. Figure 9d displays mass spectra from T 1.5 giving 7 folic acid monomer dominated by monomer with MW 442.4175 Da. and m/z range 441.95 – 443.11, and relative intensity 100%. Figure 9f shows mass spectra m/z from T 1.5 yielding 7 folic acid monomer dominated by 2 monomer with MW 442.4774 and 442.8006 Da. and m/z range 441.94 – 442.94, and relative intensity 100%.

Figure 9. (a) Chromatogram and (b) mass spectra of standard folic acid, (c) chromatogram and (d) mass spectra of instant infant porridge, (e) chromatogram and (f) mass spectra of pour infant porridge at optimum condition.

3.7. Particle size distribution

Particle size distribution from instant infant porridge shows particles with diameter size (Ø) in the range of 5.122 – 10.097 µm (< 10.00 µm) with quantity 3% or average Ø 10.27 µm with quantity 10% per volume of material, Ø ranged from 11.565 – 890.0 µm (< 100.00 µm) with quantity 50% and Ø ranged from 11019.515 – 5000 µm (< 5000 µm) with quantity 90%. In other words, particles of instant infant porridge
Porridge are found 100% per volume of material with Φ ranged from 5.122 – 5000 µm or in the whole diameter sizes (Φ) are dominated by particles with Φ 124.7022, as shown in Figure 10a. Particle size distribution in pour infant porridge displays particles with Φ in the range of 4.472 – 10.097 µm (< 10.00 µm) with quantity 4.6% or average Φ 9.38 µm with quantity 10% per volume of material, Φ in the 11.565 – 1019.515 µm or average Φ 28.54 µm with quantity small than 100.00 µm with quantity 50%, and Φ ranged from 2009.68 to 5000 µm (< 5000 µm) or average Φ 295.45 µm with quantity 90% or the whole Φ are dominated by Φ of 126.63 µm, as demonstrated in Figure 10b.

![Figure 10](image)

**Figure 10.** Distribution of particles size from (a) instant infant porridge and (b) pour infant porridge in optimum condition (fortificant A and mocav flour 18% w/w basic porridge formula).

Table 4 shows particle size and particle size distribution in both instant and pour infant porridges from the optimum formulation condition. Difference in particle size and particle size distribution from both kinds of porridges are related with texture, in which instant infant porridge is dry granule due to result of dry mixing process with average particle size of 126.63 µm. Meanwhile, pour infant porridge is suspension like pasta produced by pouring instant infant porridge by boiled water causing average particle size in pour porridge (126.63 µm) being larger compared with average particle size in instant porridge (124.70 µm) because of its occurrence of gelatinization process. The particle size distribution of instant infant porridge indicates larger particle size compared with each quantity (q) of pour instant porridge, as shown in Table 4.

**Table 4.** Particle size and particle size distribution in both instant and pour infant porridges at the optimum formulation condition by adding mocav as carrier of folic acid and thickener.

| Kinds of porridge*       | Diameter of particle size on cumulative (%) | Σ (µm) |
|--------------------------|--------------------------------------------|--------|
|                          | q (%)                                     | Undersize (µm) |
| Instant infant porridge  | 10.00                                     | 10.27  |
|                          | 50.00                                     | 71.80  |
|                          | 90.00                                     | 323.82 |
|                          | Σ                                          | 124.70 |
| Pour infant porridge     | 10.00                                     | 9.38   |
|                          | 50.00                                     | 28.54  |
|                          | 90.00                                     | 295.45 |
|                          | Σ                                          | 126.63 |
4. Conclusion

Mocav flour has potential use as food additive functioning as carrier of folic acid and thickener in preparation of instant infant porridge for complementary breast feeding (CBF) using fortificant of natural folic acid. Mocav flour concentration becoming more and more increase will increase composition of instant infant porridge fluctuate to optimization condition achieved. Based folic acid, optimization condition is achieved for fortificant A at mocav concentration 18% (w/w basic porridge formula) yielding instant infant porridge and pour infant porridge with composition of folic acid 689.73 and 445.76 µg/mL, dissolved protein 4.54 and 0.66 mg/mL, total solids 91.64 and 9.91%, total sugars 352.17 and 120.87 mg/mL, and reducing sugars 37.38 and 27.82 mg/mL, respectively. In this optimum mocav concentration, formulation system is able to increase folic acid 532.26% (5.3-folds), dissolved protein 226.62% (2.27-folds), total sugars 120.44% (1.2-folds), however decrease total solids 4.61% and reducing sugars 14.94% in instant infant porridge compared with each components concentration in base formula of infant porridge. In this condition, instant infant porridge gives dominant folic acid monomer with MW 442.4175, whereas pour infant porridge gives 2 folic acid monomers with MW 442.4774 and 442.8006 Da., and relative intensity 100%. Volatile compounds from instant infant porridge and pour infant porridge are dominated by acetic acid compound and its derivative (Thioacetic acid, methyl ester, ethyl ester) with total 44.21% and sugar compounds and its derivative (as Furfural, 2-Furanmethanol, 2 (5H)- Furane, 2H- Pyran, 4H- Pyran-4-one, Tetrahydro-4H-pyran, 5-Hydroxymethylfurfural) with total 29.812%. Particle distribution in instant infant porridge showing 10, 50 and 90% per volume per weight have particle size of 10.27, 71.80 and 323.82 µm with average 124.70 µm, while particle distribution in pour infant porridge displaying 10, 50 dan 90% per volume per weight have particle size of 9.38, 28.54 and 295.45 with average 126.63 µm.

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