e-ISSN : 2621-9468

Type of the Paper (Article)

Proximate analysis of dairy-free, ready-to-use complementary food developed with MOCAF, mung bean flour, and *tempeh* flour

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Abstract

Cow's milk is a common ingredient added to commercial complementary foods (CCFs) as a source of protein. Despite that, 2–7.5% of Indonesian infants are estimated to have cow's milk protein allergy (CMPA) and are at risk of deficiencies in protein, fat, and micronutrients due to avoiding dairy-contained CCFs. This study aimed to develop a dairy-free, ready-to-use complementary food (RUCF) by utilizing plant-based protein sources. *Tempeh* flour, mung bean flour, modified cassava flour (MOCAF), sugar, inulin, micronutrient mix, and palm oil were mixed in a low-speed Stephan mixer at 75°C for 15 minutes. The ratios of *tempeh* flour (TF) and mung bean flour (MBF) were 12:20, 16:16, and 20:12 (w/w). Proximate and micronutrient (vitamin B12, calcium, and zinc) contents were determined and compared to the standard regulation of complementary food by the Indonesian Food and Drug Association (PerBPOM) No. 24/2019. There was no significant difference between the three formulas for energy, moisture, total carbohydrate, and total fat contents ($p > 0.05$). The formula with a TF-to-MBF ratio of 20:12 had significantly higher ash and protein contents compared to other treatments ($p<0.05$). In comparison to the standard nutrient content, the fat content of all formulas exceeded the maximum standard of 4.5 g/100 kcal. Furthermore, vitamin B12 content was lower than the minimum standard of 0.05 µg/100 kcal. Although all formulas majorly fulfilled the regulatory range of nutrient content for CCF, product reformulation is necessary to adjust fat, vitamin B12, and other nutrients that has not fulfilled the regulations.

Article History
Received January 3, 2024
Accepted June 25, 2024
Published June 30, 2025

Keywords
Complementary Food, Dairy-Free, MOCAF, Mung Bean Flour, Ready-to-Use, *Tempeh* Flour.

1. Introduction

Complementary food (CF) is the staple of an infant's diet to meet both macro- and micronutrient requirements. It is commonly prepared in the form of porridge, puree, or other soft foods. The Indonesian Food and Drug Authority, or *Badan Pengawas Obat dan Makanan* (BPOM), strictly regulates commercial complementary foods (CCFs) in Indonesia, ensuring nutritional completeness in every product. Most CCFs are available in the form of powder or various flour-based products, such as pasta, which still require preparation to make the products ready to consume. In addition to these products, more convenient Ready-to-Use Complementary Foods (RUCF) are also commercially available. They are available as dry solid foods, such as baby biscuits. The market for RUCF remains underexplored, as there are limited novel alternatives to biscuits, that are not suitable for consumption by infants under the age

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ISSN 2621-9468 online. DOI 10.20956/canrea.v8i1.1230

of 12 months (1). Currently, the most feasible ready-to-use food products for infants are pastes. The utilization of paste-form food for infants has been successfully demonstrated in ready-to-use supplementary foods (RUSF) and ready-to-use therapeutic foods (RUTF) worldwide, which are primarily intended for treating malnutrition (2).

CCFs mainly comprise unique combinations of ingredients, including grains, fruits, vegetables, and animal products, to achieve various desirable traits and nutritional benefits (3). Among the commonly used ingredients, milk powder is the most widely utilized because of its desirable quantity and quality of proteins, fats, and micronutrients (4). However, approximately 2-7.5% of infants in Indonesia suffer from cow's Milk Protein Allergy (CMPA) (5). For this vulnerable group, avoiding dairy-based CCFs could lead to other nutrient deficiencies, such as protein, calcium, fat, phosphorus, and vitamin B12. Consequently, infants suffer from a diverse range of conditions including impaired growth and increased risk of obesity (6,7).

Dairy-free baby food formulations often incorporate animal protein flours (e.g., quail eggs, tilapia, and snakehead fish flours) to enhance the protein content (8,9). Alternatively, plant protein sources such as red bean and *tempeh* flours are frequently combined with carbohydrate flours (e.g., cassava and banana flours) to meet nutritional needs (10–13). This study used modified cassava flour (MOCAF), *tempeh* flour (TF), and mung bean flour (MBF) as primary ingredients. MOCAF has gained popularity owingto its relatively higher iron content than cassava flour and its significant reduction in the antinutrient phytic acid (14). However, this carbohydrate source lacks protein, making mung bean and *tempeh* flours crucial to the formulation. *Tempeh* flour is an excellent source of protein and fat, and notably provides vitamin B12, which is typically absent in plant-based ingredients. Mung bean flour balances the high-fat *tempeh* flour and prevents excessive fat in the product, while still providing substantial protein. In addition, *tempeh* flour and MOCAF complement each other's limiting amino acid profiles methionine, and lysine, respectively (15). Mung bean flour further enhanced the amino acid composition. While *tempeh* and mung bean flours contain trypsin inhibitors, tannins, phytic acid, and hemagglutinin, heat treatments (e.g., boiling, autoclaving, and microwave heating) effectively reduce these antinutrients (16).

RUCF development in this study is a new product development for a new-to-the-world product category. By adapting RUTF forms and changing the nutrition aspect to satisfy the optimal nutrient requirement, a new form of RUCF can be the next innovation in the CCF industry. In the initial stages of the RUCF development, the priority was to create a formulation that satisfied energy, macronutrient, and micronutrient requirements with acceptable viscosity. The effect of product processing on micronutrient content was also assessed to evaluate the suitability of production

2. Materials and Methods

2.1. Study Design

The study used a factorial randomized design with three replications, adjusting the amounts of TF and MBF in different ratios to determine their influence on the proximate content and viscosity of the product. Micronutrient analyses were conducted on the formulation that exhibited the best viscosity to evaluate the effect of such treatments on the micronutrient content of RUCF. The results were then compared with the existing literature and available regulations to determine the overall acceptability and quality of RUCF. As a newly developed product, lipid-based RUCF lacks well-defined parameters and comparison criteria.

2.2. Materials

MOCAF (Keola, Indonesia), *tempeh* flour (CV. Sanfood Indonesia, Banten, Indonesia), and mung bean flour (PT. Caracas Global Mandiri, Tangerang, Indonesia) were purchased from local suppliers. Palm oil (Bimoli, PT Salim Ivomas Pratama Tbk (SIMP), Jakarta, Indonesia) and icing sugar (Rose Brand, Jakarta, Indonesia) were obtained from commercial sources. Inulin (Orafti, PD Anugerah Tangerang, Indonesia), which is certified to contain 92.7% inulin and 7.3% sugar, was purchased from a commercial distributor. The micronutrient mix, containing 0.01 mcg of B12 vitamin, 4.66 mg of zinc, 423.21 mg of calcium (per 3.5 g), was supplied by a commercial partner (BPOM certified, PT. Global Vita Nutritech, Karawang, Indonesia). The heating and mixing processes were performed using a Stephan mixer equipped with an emulsifying compartment. laboratory-grade tools were used for the analysis. All the reagents and materials were obtained from the facilities of the Indonesia International Institute for Life Sciences (i3L).

2.3. Formulation

Formulation and ingredient selection for dairy-free complementary foods considered various factors, including dietary needs, government regulations (Regulation of the Food and Drug Authority (PerBPOM) Number 24 of 2019) (17), ingredient quality in comparison to skim milk, similar product developments, and consecutive processing. The formulations in Table 1 were expected to achieve the targets for each macronutrient and micronutrient content.

Table 1. Dairy-free RUCF formulations.

Materials	Formulations		
	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)
MOCAF	15.0	15.0	15.0
Tempeh flour	12.0	16.0	20.0
Mung bean flour	20.0	16.0	12.0
Palm oil	28.0	28.0	28.0
Icing sugar	14.5	14.5	14.5
Inulin	7.0	7.0	7.0
Micronutrient mix	3.5	3.5	3.5
TOTAL (%)	100.0	100.0	100.0

2.4. Food Processing

MOCAF, TF, and MBF were sieved through a 100-mesh sieve prior to processing. All ingredients were prepared and weighed using an analytical scale according to the experimental formulations and were scaled up to batches of 2 kilograms. All the ingredients were mixed simultaneously using a Stephan mixer. The mixer was set at 300 rpm, heated, and maintained at a consistent temperature of 75±2°C for fifteen minutes. The consecutive packaging process was conducted in a laminar flow machine, providing UV and airflow to ensure aseptic handling. The resulting heated paste was scraped off with a baking spatula, hot-filled into standing aluminum pouches, and stored in a cool and dry environment prior to subsequent analyses. The production of RUCF was conducted in triplicate for each formulation.

2.5. Proximate Analysis

Proximate analysis was conducted in three replicate to determine the total carbohydrate, total fat, protein, ash, and moisture contents (18). The total carbohydrate content was estimated by subtracting the protein, fat, ash, and moisture contents. Total fat content was determined using the Soxhlet method (19). Titrimetric analysis (18-8-31/MU/SMM-SIG) for protein quantification was done with the service of an external vendor (Saraswanti Laboratory Bogor). Moisture content was determined using a rapid moisture analyzer apparatus in line with AOAC Official Method 935.29. The ash content was determined using the gravimetric method according to AOAC Official Method 920.153. Finally, the energy content was calculated using the Atwater factor conversion by multiplying the amount of carbohydrates and protein by 4 kcal/g and fat by 9 kcal/g (20).

2.6. Viscosity Test

A viscosity test was conducted to determine the formulation with the best consumption-suitability traits that were further tested for micronutrient content and stability after the heating process. The analysis focused on one formulation owing to budget limitations for conducting micronutrient analysis on all three formulations. The viscosity was examined using a Lamy Rheology B First One Touch viscometer equipped with an L3 spindle. The RUCF samples were transferred into a 200 ml beaker, and the analysis was performed at rotational speed of 5 rpm for 5 min. The choice of 5 rpm for 5 min was based on the best practices in the equipment manual and was consistent with the recommended low shear rate testing for infant complementary foods. Low rotational speeds (corresponding to shear rates in the 0.1–100 s^{-1} range) better reflect the shear conditions during infant oral processing and swallowing (21–23). Lower viscosity (generally <3 Pa·s or ~3000 cP) at these shear rates has been associated with improved swallowing ability and higher nutrient intake in infants (22,23). Viscosity measurements were performedin triplicates for each formulation.

2.7. Micronutrients Analysis

Micronutrient content quantifications for vitamin B12, calcium, and zinc were done with the service of an external vendor (Saraswanti Laboratory Bogor). The analysis was performed using the best formula before and after the heat treatment. The best formulation refers to the product with the most acceptable viscosity. Each food sample from each replicate was sampled twice for duplicate analyses. Calcium and zinc levels were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), and vitamin B12 levels were analysed using Liquid Chromatography Mass Spectrometry (LC-MS/MS) methodologies. The details of the methodology are not shared with the public.

2.8. Statistical Analysis

Each proximate analysis parameter was processed with RStudio software using a one-way ANOVA test and Tukey's HSD post-hoc test to determine whether there were significant differences between each formulation and the BPOM regulation. The results are presented as the mean and standard deviation.

3. Results and Discussion

3.1. Proximate Content

The proximate analysis results in Table 2 shows no significant statistical difference between all formulations for moisture, carbohydrate, and fat contents ($p > 0.05$). A significant difference was found only in the ash and protein contents ($p < 0.05$). The data were presented in grams per 100 kcal to ease interpretation when comparing the results to the standard nutrition content of CF for children aged 6–12 months, as regulated by the Regulation of the Food and Drug Authority (PerBPOM) Number 24 of 2019 (17). In comparison to the regulation, the moisture and protein contents of all formulations were within the permissible range, although the protein content fell in the lower range of the regulation. The fat content was revealed to be the main concern since all formulations exceeded the regulation maximum of 4.5 g/100 kcal. The amounts of ash and carbohydrate were not specifically regulated.

Table 2. Proximate content of RUCF per 100 kcal for each formulation.

Proximate Composition	Regulation (PerBPOM No. 24/2019)	Formulations		
		F1	F2	F3
Moisture (g)	max. 5	0.03 ± 0.00[a]	0.08 ± 0.08[a]	0.04 ± 0.00[a]
Ash (g)	not available	0.65 ± 0.01[a]	0.67 ± 0.01[a]	0.70 ± 0.02[b]
Carbohydrate (g)	not available	10.85 ± 0.40[a]	10.62 ± 0.47[a]	10.11 ± 0.87[a]
Protein (g)	min. 1.9 - max. 5.5	2.00 ± 0.06[ab]	2.03 ± 0.08[bc]	2.12 ± 0.07[c]
Fat (g)	max. 4.5	5.40 ± 0.19[a]	5.49 ± 0.18[a]	5.67 ± 0.41[a]

Note: Each formulation result was expressed as the mean ± SD of 3 replications and 3 repetitions (n = 9), except for protein content with only two repetitions. Different alphabet letters assigned indicated significant statistical differences among formulations, as shown by Tukey's HSD post-hoc test (P<0.05).

3.1.1. Moisture

Water content is one of the most crucial parameters in RUCF, as it determines the shelf life and quality retention of the products. Although water activity is a better indicator of overall storage quality, water content is still a viable indicator, especially with available regulations (24). In this study, the moisture content in all formulations is below the permissible maximum of 5 g/100 kcal product, ranging from 0.03 ± 0.00 g/100 kcal (F1), 0.08 ± 0.08 g/100 kcal (F2), and 0.04 ± 0.00 g/100 kcal (F3) with no significant statistical difference ($p > 0.05$). This finding indicates that the variation in the TF and MBF quantities does not significantly affect the moisture content . The extremely low water content is even lower than that of other RUCF alternatives, such as baby biscuits, in the range of 1.5% to 2.5% moisture per 100 g mass of product (25). This is achievable because of the utilization of all ingredients in the form of dried powder, other than liquid oil. This suggests that product development was successful in creating a fluid texture without the addition of water.

3.1.2. Ash

The ash content of F1 to F3 was observed at 0.65 ± 0.01, 0.67 ± 0.01, and 0.70 ± 0.02 g/100 kcal in respective order. Statistical analysis indicated a significant difference in F3 compared to the other formulations ($p < 0.05$). The ash content increases with higher TF substitutions over MBF, which contradicts the findings that MBF contains a higher ash level than TF (26,27). This finding indicates variation in the actual nutritional content of each ingredient according to their respective databases and literature. Although not individually measured, ash content is a viable indicator of mineral content in food (28). Therefore, F3 could potentially contribute more to the mineral needs of infants.

3.1.3. Carbohydrate

Carbohydrates content average decreases with increasing TF substitutions, with 10.85 ± 0.40, 10.62 ± 0.47, and 10.11 ± 0.87 g/100 kcal for F1, F2, and F3, respectively. However, there were no statistically significant differences among the formulations ($p > 0.05$). Carbohydrate estimation may not be very reliable, because it is calculated indirectly from the results of other analyses. In particular with the fat content showing less than expected values, the carbohydrate content may be overestimated if the other proximate compositions contribute to additional underestimation. Therefore, proper examination of total carbohydrate content is recommended in future research to obtain more accurate comparisons with the recommended dietary allowance (RDA). The analysis of fructose or other sugar compounds could be added to evaluate whether the carbohydrate content of RUCF completely satisfies the BPOM regulations.

3.1.4. Protein

A significant statistical difference was found for protein content in F3 (2.12 ± 0.07 g/100 kcal) compared to F1 (2.00 ± 0.06 g/100 kcal) ($p<0.05$), but not F2 (2.03 ± 0.08 g/100 kcal) ($p>0.05$). The statistically significant difference between F1 and F3 indicated that a higher ratio of TF and MBF affected the protein content in RUCF and indicates the relevance of TF as the primary source of protein. All formulations have satisfied the minimum BPOM regulations for proteins at 1.9 g/100 kcal and were below the maximum recommended concentration of 5.5 g/100 kcal. Translating this trend, using a lower TF:MBF ratio in the formulation could lead to insufficient protein content in the RUCF samples. Therefore, a minimum of 12 g/100 g TF in RUCF formulation as the primary protein source would provide the minimum sufficient protein content in a commercial RUCF if appropriately complemented by other secondary ingredients. The incremental increase in TF utilization also effectively increased the protein content of RUCF. The insignificant increases in fat content following higher TF substitution also indicated that the utilization of MBF as a secondary protein source with extremely minimal fat content is successful in achieving its intended purposes.

Protein is a crucial driving factor in the growth and development of infants. The consumption of higher amounts of protein is strongly associated with an increased growth rate. Consequently, higher protein intake in infants is associated with an increased risks of obesity and being overweight in the later stages of child development (29). In addition, younger infants with extremely high protein intake could suffer a temporary and reversible hypernatremic dehydration condition where their kidney filtration rate can not handle the high renal solute load (30). These conditions are common in infants who include a higher amount of cow's milk in their diet. However, the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee stated that there is insufficient medical evidence to suggest a tolerable upper intake level (UL) of proteins for infants (31). Therefore, the protein gained from regularly consuming the developed RUCF would not pose any significant health effects on infants' health, especially for the intended group of infants who could not consume dairy-based ingredients.

3.1.5. Fat

The fat content of the RUCF ranged from 5.40 ± 0.19, 5.49 ± 0.18, and 5.67 ± 0.41 g/100 kcal for F1 to F3, respectively, with no significant statistical difference ($p > 0.05$). This finding indicated that the higher utilization of TF did not affect the fat content of RUCF. However, all three formulations surpassed the BPOM maximum limit for fat content of 4.5 g/100 kcal. The high consumption of fats in children aged 6–24 months is not positively related to the increasing risk of obesity and overweight conditions in the later stages of development (32). The American Academy of Pediatrics Committee on Nutrition (AAP), WHO/FAO, and ESPGHAN have recommended the inclusion of fats in a 6- to 36-month-old infant's diet for at least 30% of the total caloric intake, with restrictions ranging from 35% to no restrictions (33). In comparison, the established UL for fat in infant formula in the United States is 6 g/100 kcal, which is still above the average fat content of the RUCF development (34). The same study also showed no evidence of a relationship between high-fat consumption in infants and any detrimental short-term and long-term health conditions. Therefore, the high fat content in the RUCF can be justified, as it is recommended for optimal growth and development in infants.

Instead of the amount of fat, the fat quality is closely associated with such conditions. This study used palm oil in its formulation, a notable source of saturated fatty acids that does not contain trans fatty acids (35). While there is a strong recommendation to limit trans fatty acids to 2% of the total energy, the literature around saturated fatty acids and dietary cholesterol restrictions in toddlers remains inconclusive (33,36,37). However, there is a need to ensure adequate intake of essential fatty acids, specifically arachidonic acid (ARA) and docosahexaenoic acid (DHA) in early infancy, as insufficient levels of these essential fatty acids can lead to alterations of neurological, immunological, and cardiological functions (37). Future attempts to reduce the fat content may include the introduction of water as an ingredient, utilization of low-fat and high-protein alternatives to TF, unsaturated fatty acid sources, or food additives such as emulsifiers and fat substitutes or replacements. Although not regulated by the BPOM, low carbohydrates in infant diets could lead to compromised energy utilization and impaired growth and development (38). Despite the high total energy, the RUCF contains more fat than the carbohydrate content, which is the primary energy fuel in infants' diets. This could likely cause the infants to derive most of their energy from fat and disrupt their metabolic activities. In addition, insufficient glucose hinders the growth, development, and activities of the glucose-dependent organs, especially the brain (39). The developed RUCF may need to be paired with a high-carbohydrate or high-glucose food alternative to supplement the potential shortage of carbohydrates provided.

3.2. Viscosity

The viscosity test indicated a significant difference between all the formulations ($p < 0.05$), with a decrease in viscosity for higher TF utilizations. As shown in Figure 1, the viscosities of F1, F2, and F3 were 82.44 ± 4.15 $mPas^{-1}$, 72.19 ± 2.97 $mPas^{-1}$, and 63.39 ± 3.18 $mPas^{-1}$, respectively. This suggested that F3 is a formulation that is more suitable for infants with less developed oromotor capabilities. Although viscosity is not a parameter that is regulated by any CCF standards, F3 was determined to be the most optimal physical property for micronutrient analysis.

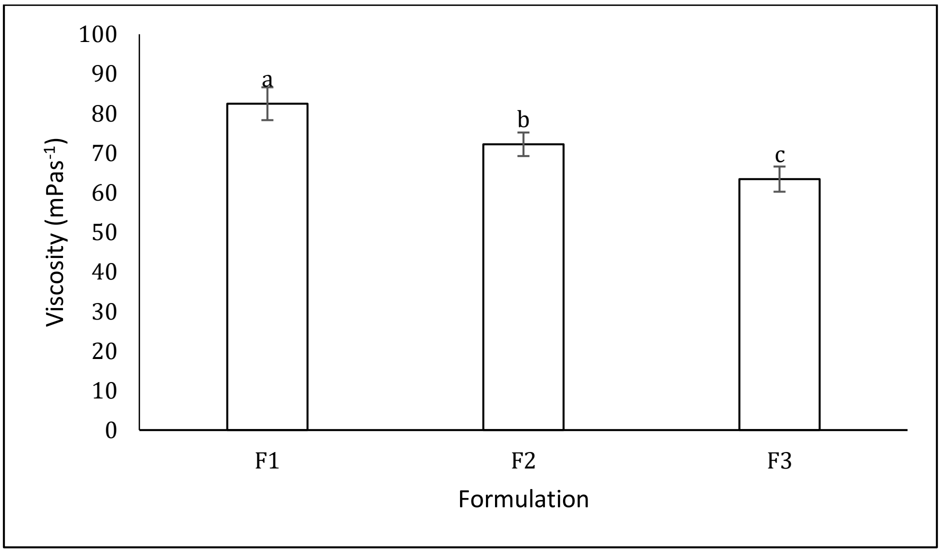

Figure 1. Preliminary Viscosity Analysis Results. Each formulation result was expressed as the mean of 3 replications and 3 repetitions (n = 9). Different alphabet letters assigned indicated statistical similarities among formulations, as shown by Tukey's HSD post-hoc test ($p > 0.05$).

Lower viscosity of complementary foods has been demonstrated to support better swallowing and increased intake among infants, particularly those with immature oromotor skills (22). Foods with lower viscosity reduce the risk of fatigue during feeding and improve the energy and nutrient delivery per meal. This study further highlighted that complementary foods with moderate viscosity enhance functional swallowing properties and acceptability in infants (23). These findings reinforce the selection of F3 as the most suitable formulation for infant consumption.

According to the descriptive observations, the developed RUCF strongly resembles category four of the International Dysphagia Diet Standardisation Initiative (IDDSI) textural properties standard, which is the level of food thickness and firmness suitable for infant consumption (40). However, the RUCF has one textural description that was not fulfilled. The RUCF does not fall off the spoon under a simple gravity test, although it does with a slight force applied as allowed. Nevertheless, the slightly higher-than-desirable stickiness of the RUCF is an indication of its potential unsuitability for infants' consumption. The IDDSI standard also provides an analytical framework that can quantitatively categorize food textures. Future studies with physicochemical property analyses could include these frameworks to determine consumption suitability and provide an initiative for formulation or processing improvements.

3.3. Micronutrient Content

Out of the three micronutrients tested, conclusions could be drawn only for calcium and zinc (Figure 2). Because the method of analysis could only detect the minimum vitamin B12 content of 0.8 µg/100 g, the actual content of vitamin B12 became inconclusive. This finding suggested that the presence of vitamin B12 before and after the heat treatment was below 0.8 µg/100 g or 0.02 µg/100 kcal. This value was lower than the minimum regulated amount for vitamin B12 content of 0.05 µg/100 kcal. However, there was a decrease in the amount of calcium and zinc after the heat treatment. Calcium decreased slightly by 4.14% from 99.08 ± 0.32 to 94.98 ± 1.04 mg/100 kcal, although it still satisfied the minimum BPOM regulation of 80 mg/100 kcal. However, the heat treatment decreased zinc content by 23.75%, from 1.00 ± 0.00 to 0.77 ± 0.01 mg/100 kcal, below the minimum BPOM regulation of 0.86 mg/100 kcal.

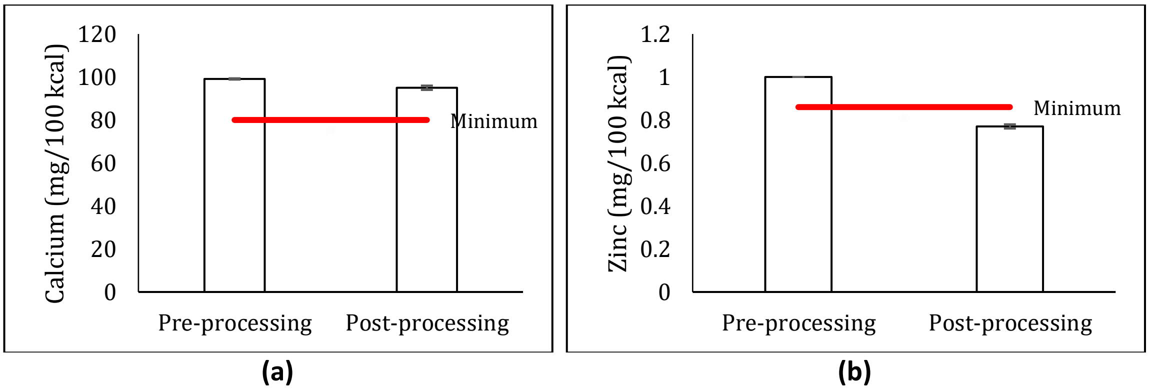

Figure 2. Micronutrient content per 100 kcal product before and after heating process (75±2°C, 15 minutes): (a) calcium content compared to BPOM regulation (indicated by red line); (b) zinc content compared to BPOM regulation (indicated by red line); The BPOM regulation is derived from the minimum micronutrient content of complementary food for children aged 6–12 months old. Each formulation result was expressed as the mean of 2 repetitions (n = 2).

TF is the only component of ingredient | that contains vitamin B12. Thus, the higher TF utilization in F3 should result in higher vitamin B12 contents among all three formulations. Therefore, the null measurement in F3also suggests that F1 and F2 do not have vitamin B12 content above the detection limit (0.08 µg/100 g). The micronutrient mix contains 0.09 µg/100 g of vitamin B12, which translates to less than 0.01 µg/100 g in the RUCF. This suggests that the micronutrient mix added and the natural vitamin B12 in the other ingredients combined do not provide equal or more than 0.08 µg/100 g. Future formulations could obtain a more customized micronutrient mix that addresses the need for higher amounts of vitamin B12, preferably at a minimum of 0.25 µg/100 g micronutrient mix (currently at 0.09 µg/100 g), assuming 3.5 g/100 g of it will be used in RUCF.

The upper tolerable intake level (UL) of calcium and zinc in CCFs is not regulated by most regulations because of the low risk of health effects of overconsumption in infants (41,42). However, UL for calcium is regulated in infant formula milk due to the extremely high calcium content (39). As a comparison, the UL of calcium content in infant formula milk is 65–75 mg/100 kcal, which is lower than the minimum requirement for CCF as regulated in PerBPOM No. 24/2019. Since CF is used less frequently than infant formula, higher tolerable levels of calcium are to be expected. Furthermore, as the RUCF is intended for infants who do not consume dairy-based ingredients, their pairing with cow's milk formula would be unlikely to occur and cause calcium over-consumptions. On the contrary, zinc's UL for infant formula is 1.5 mg/100 kcal, which is still above the average zinc content in the developed RUCF (42).

The calcium content satisfied the minimum regulation of 80 mg/100 kcal by 117-119%. By comparing the amounts in pre-heat treatment RUCF at an average of 531.52 mg/100g, the estimated 423.21 mg/100 g in the micronutrient mix used contributed to approximately 79.6% of the total calcium in pre-heat treated RUCF. This suggests that without the micronutrient mix, the natural calcium content from the other ingredients in F3 would only satisfy about 20% of the regulation. On the other hand, the RUCF can only fulfill 88% to 90% of the higher regulations at 0.86 mg/100 g for infants in the 0-6 month group. Calcium is a relatively heat-stable mineral that is not affected by either light or reactive metals (43). It is more stable than zinc and aluminum, therefore unlikely to interact with the pasteurization and packaging processes that involve aluminum and more stable metals (44). As indicated by the micronutrient analysis results, calcium undergoes very minimal losses after the whole RUCF processing. Therefore, the calcium content in the micronutrient mix can withstand pasteurization, UV-aided hot filling, and prolonged storage in aluminum-based packaging.

The amount of zinc found in the RUCF products after processing was greatly reduced compared to calcium. Zinc has evidently been one of the most easily lost during food processing, especially when heat treatments are involved (45). In addition, zinc is a reactive metal that could easily interact with more reactive metals, such as aluminum and iron, in packaging materials and cookware (44). These interactions usually result in minerals being lost by their deposition and sticking to metal surfaces. Therefore, zinc loss in production could mainly be caused by the constant mixing process that enables zinc to stick to the metal cookware surface. Discounting the losses from heat treatments, 4.66 mg of zinc was added to the micronutrient mix, accounting for 86% of the zinc found in the pre-treatment RUCF. These are as expected since plant-based ingredients do not provide adequate amounts of calcium and zinc that are usually found in animal-based ingredients (46). Therefore, despite the potential for significantly different calcium and zinc contents in different TF:MBF ratios that are not measured in this study, dairy-free RUCF development would need to heavily rely on fortifications to enhance their calcium and zinc contents. The zinc content in the micronutrient mix could be slighty increased y in future formulations to account for potential losses during processing.

3.4. Study limitations

Despite these substantial findings, this study had several limitations. Owingto time and resource constraints, this study focused specifically on macronutrient content and the potentially limiting micronutrient content. In addition to the nutrients that were the focus of this study, the Indonesian Food and Drug Authority also regulates the requirements for other nutrients, such as dietary fiber, omega-6 and omega-3 fatty acids, energy density, and other vitamins and minerals. Examining these nutrients and exploring alternative ingredients is crucial for enhancing the quality of RUCF. In addition, achieving the desirable viscosity is also important to ensure that the product is suitable for young children whose eating abilities are not yet well-developed. Finally, to ensure safety and acceptability, various analyses such as microbiological analysis and sensory evaluation could also be employed to satisfy the standard regulations for complementary foods.

4. Conclusions

The objective of developing a dairy-free RUCF that completely satisfies BPOM regulations has not been fully achieved, with excessive fat content and insufficient zinc and vitamin B12 contents in all three proposed formulations. The moisture, protein, and calcium contents were well within the regulation, while other parameters such as total energy, carbohydrates, and ash content were not strictly regulated. F3, with 15% MOCAF, 20% *tempeh* flour, and 12% mung bean flour, is subjectively determined as the best formulation with the best overall proximate content (higher protein with less significantly higher fat) and the best overall viscosity test result and could serve as a reference for future developments. Calcium and zinc stability is compromised during RUCF production, especially for zinc.

The current RUCF formulations may benefit infants better with controlled consumption. For example, pairing up a serving of RUCF with high-carbohydrate meals throughout the day would minimize the risk of overconsumption of protein, fat, and micronutrients. Alternatively, dividing a portion of the RUCF to complement other meals as a nutritional booster could be an attractive feeding practice. The RUCF product should be reformulated in future studies to improve its nutrient contents. Utilizing more carbohydrate source and sugar, experimenting with food additives such as fat replacers or emulsifiers, and using lower-fat-content alternative ingredients can make the product more suitable for regular consumption. Obtaining a customized micronutrient mix could fulfill every micronutrient regulation with the minimum amounts, allowing more utilization of other ingredients.

Acknowledgements

We extend our heartfelt gratitude and appreciation to the esteemed Indonesia International Institute for Life Sciences (i3L) for generously granting us an invaluable opportunity to conduct our research within their state-of-the-art asset laboratories. This study would not have been possible without unwavering support and cooperation . Their commitment to advancing scientific exploration has been instrumental in the success of our research. We sincerely acknowledge their indispensable contributions, which have significantly improved the quality and scope of our work.

Author Contributions

D.D., W.I., and R.M.S.I conceived and designed the experiments; D.D. performed the experiments. All authors analyzed the data, prepared the original draft, reviewed, and edited the final manuscript.

Funding

This research was funded by the Indonesia International Institute for Life Sciences (i3L) through an internal research grant.

Institutional Review Board Statement

Not applicable.

Data Availability Statement

The data supporting the findings of the article is available within the article.

Conflicts of Interest

All authors declare no conflict of interest, financial or otherwise.

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