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HIGH-PROTEIN COMPLEMENTARY FOOD POWDER BASED ON FERMENTED COW'S MILK WITH COMPOSITE FLOUR

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ABSTRACT
Adequate nutrition for infants during crucial periods is crucial for their development and growth. Providing nutrient-dense complementary foods (CF) and probiotics is crucial for infants, not only for high protein intake but also for the addition of probiotics in CF to maintain digestive tract health and improve nutrient absorption. This study aims to evaluate the nutritional and organoleptic characteristics of the development of a high-protein and probiotic CF formula with composite flour (corn, red bean, and soybean) and cow's milk curd flour. The best treatment will be tested for amino acid profiles. The method is experimental with a Completely Randomized Design (CRD) consisting of 4 treatments and 4 replications. Data were collected laboratory proximate analysis using Soxhlet, and Kjeldahl, organoleptic data using hedonic tests with panelists through structured questionnaires, and amino acid profiles were analyzed using High Performance Liquid Chromatography (HPLC). The water content ranged from 8.29% - 8.75%, the ash content ranged from 3.65% - 3.90%, the fat content ranged from 17.08% - 20.24%, the protein content ranged from 17.77% - 19.21%, and the carbohydrate content ranged from 48.12% - 52.87%. The organoleptic test results for formulas F3 and F4 were most acceptable to the panelists based on the results of sensory parameters. The highest essential amino acid profiles were methionine and isoleucine while the non-essential amino acids were glutamic acid and aspartic acid. The addition of cow's milk curd flour can increase protein and fat while the addition of composite flour increases carbohydrates.

Keywords: composite flour; complementary food; fermented milk flour; food powder; high protein

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INTRODUCTION

Nutritional adequacy during the 1000 HPK period, which includes 270 days in the womb and up to 730 days after birth, is crucial. During that period, a high intake of nutrients and calories was needed to support the rapid growth and development taking place. Insufficient nutritional needs at that age will contribute to nutritional problems leading to impaired cognitive and motor development ("Nutrition Guidelines of Complementary Feeding for Infants and Toddlers," 2023). Inadequate complementary feeding (CF) can lead to nutritional problems for infants. This will risk the occurrence of stunting (Ramadani & Muniroh, 2022). The selection of raw materials can optimize the formulation of protein-source raw materials in CF to obtain the highest protein content (Putri et al., 2019). CF with high protein quality can be produced by selecting local food-based ingredients. One way is to use composite flour made from corn flour, soybean flour, and red bean flour, with the addition of curd flour, which is obtained from milk fermentation using Lactic Acid Bacteria (LAB). Dadiah, or "Dadih," a yogurt-like product fermented from buffalo milk originating from West Sumatra. The fermentation process of dadih results in a product high in protein, healthy fats, and probiotics. However, due to the limited availability of buffalo milk compared to cow's milk, dadih flour was produced. Dadih flour, a product development from cow's milk with buffalo milk starter, can be a more economical and easily accessible alternative without reducing its nutritional benefits (Helmizar et al., 2022). The nutritional composition of dadih flour is standardized according to the recommended nutrient requirements for children under two years of age (Helmizar et al., 2020).

Adding probiotics to the composition of complementary feeding ingredients provides excellent benefits for the baby's health. Probiotics have various benefits for digestive health, particularly in boosting the body's immunity by maintaining the intestinal barrier and mucosa and regulating permeability. This will improve digestion, thereby increasing nutrient absorption. Additionally, it can reduce gastrointestinal disturbances such as colic, bloating, and diarrhea, which are common in infants (Das et al., 2022; Wang et al., 2021). Giving probiotics in the form of yogurt is safe for infants and can even support their growth (Jannat et al., 2023). Composite flour has better nutritional value, especially in terms of protein, compared to flour made from a single type of food. Composite flour mixtures can provide balanced nutrition, thus improving product quality in food product development (Hasmadi et al., 2020). Soybean flour, red beans, and corn are a combination of three food ingredients that have the potential to provide good quality nutrients to meet the nutritional needs of infants.

The glutamate content in corn is the highest, followed by leucine, alanine, and proline, which together contribute over 50% of the total amino acid content in corn. Corn is a source of carbohydrates, although its protein content is relatively low. The amino acid composition of fresh corn is close to the FAO/WHO standard, it has high digestibility and absorption efficiency, and it has the potential to meet human amino acid needs (Li et al., 2022). Soybeans and kidney beans are good, inexpensive, and easily processed sources of protein for complementary feeding. Soybeans contain the highest protein content (35%–42%) compared to other beans, and about 3% lecithin, which is beneficial for brain development, especially in infants. In addition, red beans are a type of legume that contains around 22.7% protein, and is rich in vitamin B and minerals (Qin et al., 2022; Rosiana et al., 2023). Additionally, kidney beans are a type of legume that contains approximately 22.7% protein and are rich in B vitamins and minerals. However, despite their high nutritional value, soybeans and red beans contain several antinutrient compounds that can inhibit the absorption of protein and minerals. In the process of making this complementary feeding, various processing techniques are used, such as soaking, boiling, and germination, which can reduce the levels of these antinutritional compounds, thereby improving the digestibility of the product (Heuvey & Ahure, 2024).

The development of this complementary feeding product made from local food ingredients is based on cow's milk curd flour with composite flour (corn, soybeans, and red beans). This material is high in protein and complementary amino acids. Additionally, the addition of whey flour, which is used to maintain the digestive health of infants, can improve digestion in infants and prevent gastrointestinal disorders. This research aims to examine sensory acceptance, nutrient composition, and amino acid content of the best formula. This research aims to apply high-protein complementary feeding formulas made from cow's milk curd flour and composite flour (corn, red beans, and soybeans) to provide a high-protein complementary feeding alternative containing probiotics.

METHOD

The ingredients used in making complementary foods for infants and young children consist of composite flour from corn, soybeans, and red beans. Next, the flour ingredients are mixed with cow's milk curd, skim milk, powdered sugar, and palm oil. Cow's milk curd flour is made from fermented curd that is dried using the spray drying method. This formulation is determined based on an estimated calculation of the total nutritional content of the raw materials for complementary feeding, considering the energy, protein, carbohydrate, and fat levels based on instant powdered complementary feeding according to SNI 01-7111.1/2-year 2005.

The equipment for making composite flour includes a blender, an 80-mesh sieve, and a "Kirin" oven. Time and Place of Research This research was conducted from May to November 2025. Proximate and amino acid tests were conducted at the Vahana Scientific Laboratory in Padang. Subsequently, organoleptic tests were performed at the Food Science Laboratory, Department of Nutrition, Faculty of Public Health, Andalas University. The research included the production of composite flour and the mixing of all CF raw materials. The composite flour consisted of corn flour, soybean flour, and red bean flour in a 1:1:1 ratio. The treatments were given at four levels, namely the ratio of composite flour to cow's milk curd flour. The treatments are shown in Table 1.

Table 1.
Formulation of Composite Flour and Curd Flour for Complementary Feeding

Ingredients	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Composite Flour	60	55	50	45
Skim Milk	20	20	20	20
Cow's Milk Curd Flour	5	10	15	20
Powdered Sugar	5	5	5	5
Palm oil	10	10	10	10

Preparation of Complementary Food Samples
The process of making composite flour from each ingredient is processed separately until it becomes flour. Soybeans, red beans, and corn kernels were washed and soaked in clean water for 12 hours. Then, they were boiled for 20 minutes until tender enough. The boiled ingredients were drained for 15 minutes. Dry in an oven at 55–60°C for 18 hours. Then, blend until smooth and sift the flour using an 80-mesh sieve. Soybean flour, red bean flour, and corn flour were first mixed to obtain composite flour, after which all CF ingredients were combined.

Proximate Analysis
Analysis Proximate analysis was performed using the AOAC method in 2016. For fat content measurement using the Soxhlet method, protein using the Kjeldahl method, and carbohydrates by difference. The best formula is followed by an amino acid test.

Organoleptic Testing
Organoleptic Test The resulting formulation was presented with the addition of water. An organoleptic test was conducted on 30 semi-trained panelists to determine their assessment of the hedonic and hedonic quality of the formula. The parameters tested included color, aroma, taste, texture, and overall using a scale of 1 (Dislike) to 5 (Like).

Amino Acid Analysis
Amino Acid Analysis High-Performance Liquid Chromatography (HPLC) (IK. LP-04.7-LT-1.0) technique is used as the standard for amino acid testing. HPLC consists of 4 steps: protein hydrolysate preparation, drying, derivatization, injection, and amino acid analysis.

Statistical Analysis
Proximate analysis data were subjected to one-way analysis of variance (ANOVA) using SPSS Statistics version 16.0. When statistically significant differences were detected, mean comparisons were conducted using Duncan's Multiple Range Test (DMRT) at a significance level of $\alpha = 0.05$.

RESULT
Proximate Testing
Proximate Analysis The raw materials were characterized thru proximate analysis. The following are the results of the proximate analysis performed on this CF. Based on the results of the proximate analysis in Table 2, the proximate analysis of the four MP-ASI formulas (F1, F2, F3, F4) showed that all analyzed parameters exhibited variations in nutrient content values. The highest protein content was found in formula F4 (20.24%), followed by F3 (19.36%), F2 (18.01%), and the lowest

in F1 (17.08%). Fingerprint analysis showed that adding curd flour concentration to complementary feeding had a significant effect (P<0.05) on protein content. In the DMRT test, the protein content of each treatment was significantly different from each other (P<0.05).

Table 2.
Proximate Test Analysis of MPASI Products in units of g per 100 g

Formula	F1	F2	F3	F4	SNI
Protein	17.08^{a}	18.01^{b}	19.36^{c}	20.24^{d}	15 – 22
Fat	17.77^{a}	18.56^{b}	19.02^{b}	19.21^{b}	10 - 15
Carbohydrate	52.98^{a}	51.19^{B}	49.31^{b}	48.12^{c}	-
Water	8.29^{a}	8.40^{bc}	8.64^{cd}	8.75^{d}	Maks. 4
Ash	3.90^{a}	3.83^{ab}	3.74^{b}	3.65^{d}	3,5

Note: Superscripts with different letters a, b, c in the treatment indicates a significant difference (P<0.05).

Organoleptic Test

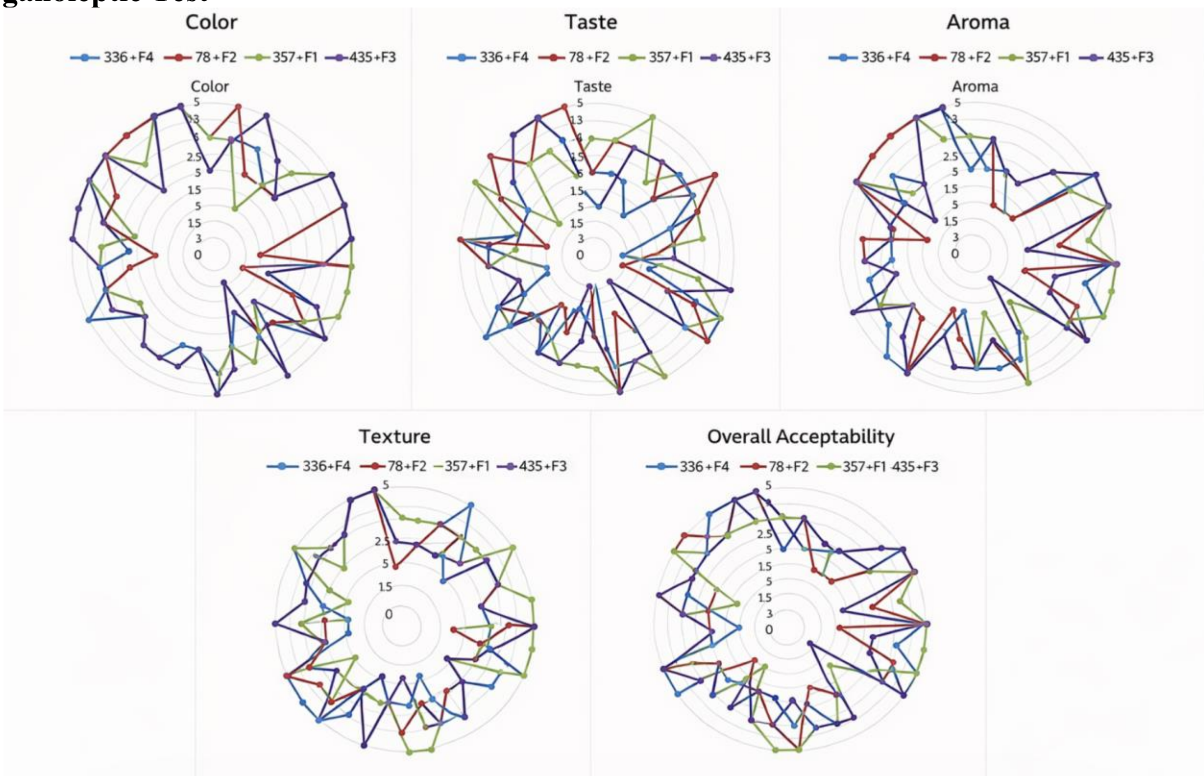

Figure 1. Spider Chart Organoleptic Evaluation of Complementary Feeding Products: Dadih Flour and Composite Flour

The results of the organoleptic test of composite flour and curd flour-based complementary feeding (MP-ASI) showed that each formula (F1, F2, F3, and F4) had different sensory characteristics for each parameter. In terms of color, F3 and F4 tend to receive more stable and higher ratings, indicating a preferred brightness and color consistency by the panelists compared to F2, which showed greater fluctuations. Regarding taste attributes, F2 and F4 appeared to dominate with scores more frequently in the medium to high range, suggesting they both provided more acceptable flavors, while F1 showing more value decline at some points.

Amino Acid Analysis

The results of the Amino Acid test showed that the highest content of essential amino acids was Isoleucine (2.30%) and Methionine (2.15%). Meanwhile, the highest non-essential amino acids were Glutamic Acid (8.69%) and Aspartic Acid (3.73%). The quality of protein in a product is determined by the composition of its constituent amino acids. Amino acids are the result of protein synthesis from food obtained thru the body's ingestion, digestion, and absorption processes. Amino acids are divided into essential and non-essential amino acids.

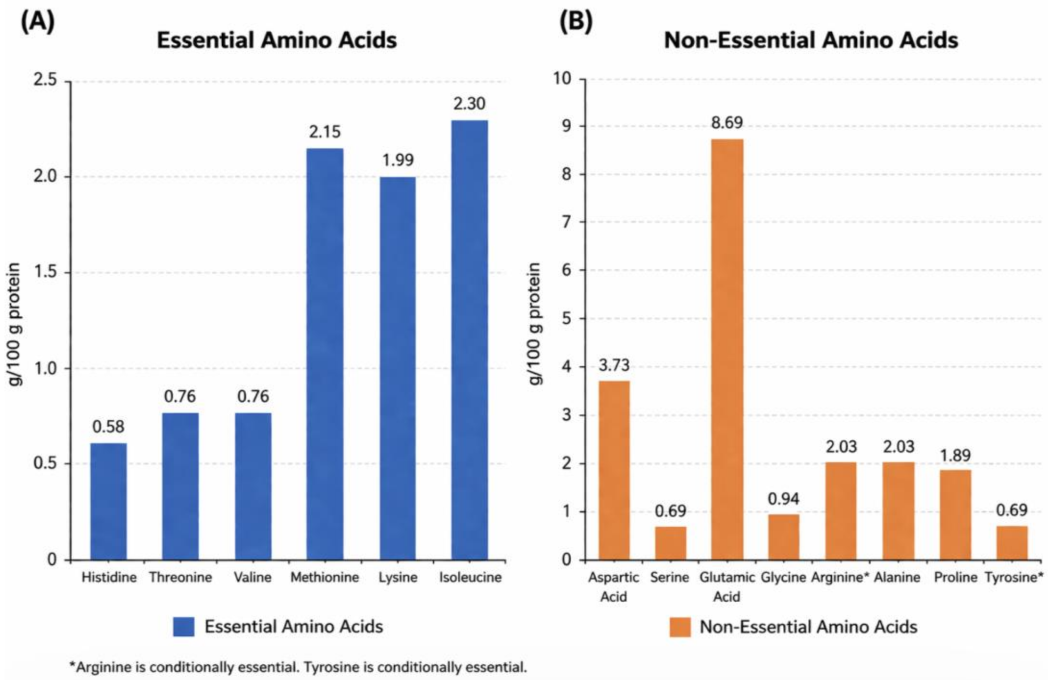

Figure 2. Analysis of Essential Amino Acids (A) and Non-Essential Amino Acids (B) in Complementary Feeding Products

DISCUSSION

Based on Table 2 the results of the proximate analysis show that modifying the formula of MP-ASI affects the composition of nutrients it contains. Protein content consistently increased from F1 to F4. This increase is generally due to the addition of protein-rich food sources such as legume flour, milk, eggs, or animal products. Regulatorily, the protein content of all formulas already meets the requirements of SNI 01-7111.1-2005 regarding CF, which is 15–22%, making it suitable as a protein source for child growth. The high protein content in this CF formula is consistent with research on making MPASI using local animal food products (turmeric fish), which has 20.53% protein with the raw material drying method using a spray dryer (Martosuyono et al., 2019; Putri et al., 2019). Spray dryer drying is often used to dry milk products into powder because it can improve the stability of milk products during storage. This drying process does not change the nutritional and sensory characteristics (Felfoul et al., 2022).

The highest fat content was also found in formula F4 (19.21%), followed by F3 (19.02%), F2 (18.56%), and the lowest in F1 (17.77%). Fingerprint analysis showed that adding curd flour concentration to complementary feeding had a significant effect ($P<0.05$) on fat content. DMRT analysis showed that the fat content of complementary feeding between treatments F1 and F2, F3, and F4 was significantly different ($P<0.05$). However, there was no significant difference between treatments F2, F3, and F4 ($P>0.05$). The fat content in all four formulas ranged from 17.77% to 19.21%, which means it all exceeded the SNI standard (10-15%). High fat content is found in formulations with the highest proportion of cow's milk curd flour. Cow's milk fat undergoes changes during the fermentation process. Short and long-chain fatty acids are formed during fermentation. Additionally, the fatty acid composition can decrease or increase due to processing methods such as heating and fermentation homogenization and storage (Sumarmono et al., 2023).

Fat is essential for babies' growth and physical activity. During growth, a higher fat intake is needed compared to adults and the elderly. Fats serve as a structural component of tissues, such as for the development of brain and nerve cells. Breast milk has a higher fat content than formula. Breastfed infants have higher total plasma blood cholesterol concentrations compared to formula-fed infants. Cholesterol is only found in animal products, but it is not present in plant-based products. Infant diets providing <22% energy from fat and low in animal fat can hinder growth. Besides providing energy and physical activity. Fat can also increase the metabolism of fat-soluble vitamins and can provide a feeling of fullness in infants (Lecerf & De Lorgeril, 2011; Martosuyono et al., 2019; Uauy et al., 2000).

The balance of gut microorganisms, achieved by increasing probiotic intake from cow's milk curd, will more effectively influence the absorption and metabolism of fats and cholesterol (Samtiya et al., 2021). The highest carbohydrate content was found in F1 (52.98%), followed by F2 (51.19%), F3 (49.31%), and the lowest in F4 (48.12%). Fingerprint testing showed that adding curd flour concentration to MP-ASI had a significant effect ($P<0.05$) on carbohydrate content. In the DMRT test, the carbohydrate content of each treatment was significantly different ($P<0.05$), namely treatments F1, F2, F3, and F4. However, treatments F2 and F3 were not significantly different ($P>0.05$).

The carbohydrate content shows a decreasing trend from F1 to F4. This generally happens because an increase in protein and fat will lower the proportion of carbohydrates in the total composition. Although SNI does not regulate carbohydrate limits, carbohydrates are the main energy source for MP-ASI. The increase in carbohydrate value is evident from the increased composition of composite flour. This complementary feeding formula has a lower carbohydrate content than complementary feeding formulas made from soybeans and dragon fruit peel, which range from 58.62-64.20% (Rosiana et al., 2023). Corn is the third most consumed source of carbohydrates by Indonesians, after rice and cassava (Daniels et al., 2023).

The highest moisture content was found in F4 (8.75%) and the lowest in F1 (8.29%). Fingerprint analysis showed that adding curd flour concentration to complementary feeding had a significant effect ($P<0.05$) on moisture content. DMRT analysis of moisture content between treatments F1, F2, and F4 showed significant differences ($P<0.05$). However, treatment F3 did not show significant differences ($P>0.05$) compared to treatments F2 and F4. Treatment F3 showed significant differences ($P<0.05$) compared to treatment F1. The moisture content in all formulas is between 8–8.7%, which means it does not meet SNI standards. High moisture content affects product stability, making it susceptible to microbial growth and shortening shelf life. This indicates that the product drying or processing is not yet optimal, so it is necessary to improve the drying process (spray dryer/low-temperature oven) to ensure safe storage.

The highest ash content was found in F1 (3.90%), followed by F2 (3.83%), F3 (3.74%), and the lowest in F4 (3.65%). Fingerprint analysis showed that adding dadih flour concentration to MP-ASI had a significant effect ($P<0.05$) on ash content. DMRT analysis showed that ash content in treatments F1, F3, and F4 differed significantly ($P<0.05$). However, treatment F2 showed no significant difference ($P>0.05$) from treatments F1 and F3. The ash content reflects the mineral content. The ash values for F1–F3 are slightly above the Indonesian National Standard (SNI), while F4 is close to the standard. High ash content can come from mineral-rich foods such as legumes, dried vegetables, or mineral fortification. Nutritionally, this is positive, but it's still necessary to consider regulatory limits.

Based on Figure 1, the aroma parameter shows a similar trend, where F3 and F4 performed more consistently, while F1 showed greater variation, indicating less stable aroma acceptance. For texture attributes, F3 appeared most stable with values tending toward the medium to high range, while F2 and F4 showed greater variation related to the perceived roughness or smoothness of the product by the panelists. Overall, in terms of overall acceptance parameters, F3 and F4 appeared to be the most positively scored formulas, reflecting a balance between color, aroma, taste, and texture that was better evaluated by the panelists. Meanwhile, F1, as the base formula, tended to have lower and less consistent values across most attributes. Overall, F3 and F4 appeared to be the most acceptable formulas to the panelists based on the overall results of the five sensory parameters. Protein contributes to the flavor of food. Most amino acids have a flavor that enhances the taste of products.

Glutamic acid and aspartic acid are umami amino acids. Alanine, glycine, serine, proline, and threonine are sweet amino acids. Bitterness come from leucine, isoleucine, valine, phenylalanine, methionine, histidine, and arginine (Li et al., 2022). Additionally, the contribution of fatty acids in cow's milk curd flour enhances the flavor and aroma of the product produced by lactic acid bacteria in yogurt cultures by converting free fatty acids into short-chain fatty acids such as butyric acid and caproic acid (Sumarmono et al., 2023).

Based on Figure 2, essential amino acids can only be obtained from food and are not synthesized by the body. There are eight types of essential amino acids for adults, while infants have nine essential amino acids because they cannot synthesize histidine (Li et al., 2022). The histidine content in this complementary food is lower compared to complementary foods made with fish meal as the raw material. The amino acid histidine plays a role in strengthening myelinated nerve cells in the child's brain, allowing for more optimal impulse transmission throughout the body and reducing the risk of mental disorders (Nurfaidah et al., 2024). Glutamate, aspartate, arginine, glycine, threonine, tyrosine, methionine, leucine, and lysine are medicinal amino acids that have therapeutic effects in maintaining nitrogen balance in the body, especially in infants, to prevent malnutrition (Li et al., 2022). The use of animal protein sources in complementary feeding. High-protein meals increase plasma amino acid concentration for several hours after eating. Plasma amino acid concentration controls the body's balance in the formation of muscle mass, hormones, enzymes, and so on, and excess protein will be stored in the form of glycogen (Loveday, 2023).

CONCLUSION

The composite flour MP-ASI formulation with cow's milk curd flour contains protein and fat. The protein and fat content increases with an increase in the composition of cow's milk curd flour. High acceptability was found in the formula with the highest curd flour composition. The most abundant essential amino acids are Isoleucine and Methionine. The highest non-essential amino acids are Glutamic Acid and Aspartic Acid. This formula for complementary feeding can be recommended for babies, especially those experiencing malnutrition.

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