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Amino acid composition and protein quality of instant liquid food based on Catfish (*Clarias gariepinus*) and Kelor (*Moringa oleifera*) flour

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Abstract. Under-five children are a vulnerable group whose susceptible to repeated infections and deficiency of protein and calcium. The objective of this research is to analyze the amino acid composition and protein quality of instant liquid food based on catfish (*Clarias gariepinus*) and kelor (*Moringa oleifera*) flour according to the requirement of children 1–3 years old. The amino acid composition was analyzed by using Ultra Performance Liquid Chromatography (UPLC). The highest essential amino acid content in the instant liquid food based on catfish and kelor flour is the 114.29±0.79 mg/g protein lysine and the highest non-essential amino acid is the glutamic acid of 244.14±1.86 mg/g protein. The protein quality was determined by the chemical score (78.30 for sulphuric amino acid), the amino acid score (100), the essential amino acid index (EAAI = 0.98), the predicted protein efficiency ratio (P-PER = 4.63±0.02), and the predicted biological value (P-BV = 48.51±0.13).The study showed that instant liquid food based catfish and kelor flour containing high-quality protein and calcium that is recommended for children aged 1–3 years.

1. Introduction
Wasting and stunting problems among under-five children in Indonesia is seriously affected health problems and the economic losses. Under-five children are a vulnerable group whose tends to suffered repeated infection and lack of protein and calcium consumption which caused a metabolic disorder that affects the children growth and development [1][2].

Proteins are nitrogen-containing substances that are formed by amino acids. They serve as the major structural component of muscle and other tissues in the body [3]. Amino acids play a central role as the building blocks of proteins and as intermediates in metabolism and further help to maintain health and vitality. There are 20 amino acids that can be found in the human body, 18 of which are important in human nutrition. Essential amino acids cannot be synthesized de novo by humans and other mammals and must be supplied in the diet. The quality of any food protein is determined by the content of essential and nonessential amino acids; the mutual proportions of specific essential amino acids; the energy supplied, which is essential for protein synthesis in the body; and the digestibility of the protein [4].

Clarias and kelor flour are an ideal source of essential amino acids. The instant liquid food modification has been developed with the addition of catfish flour (*Clarias gariepinus*) and moringa flour (*Moringa oleifera*). The protein value of instant liquid food is 15.7 g/100 g (> 35% ALG, Acuan Label Gizi, the reference of BPOM RI), so it is categorized as a high-protein food for children aged 1–3 years old. Also, it is categorized as high calcium food for children aged 1-3 years since the calcium content is 488.42 mg/100 g (> 30% ALG) [5].

Catfish (*Clarias gariepinus*) is one of Indonesia's rich animal protein sources with a high score of bioavailability and complete amino acids and fatty acids [6][7]. A research by Wibisono proved on post-surgical patients at the hospital showed the increase in energy and protein as well as prealbumin levels after five days consumed liquid food based on catfish flour intervention [8].

Kelor leaf (*Moringa oleifera*) used as a vegetable with high nutritional and health beneficial value [9]. It contains more than 90 nutritional chemical compounds, including proteins, lipids, carbohydrates and dietary fibers. It is commonly used in the tropical countries as a food source to overcome malnutrition among children and infants [10]. It has calcium with 17 times in milk and a protein equivalent to 9 times in yogurt [11]. Among the several nutrients found in different parts of M. oleifera, proteins are the most abundant, accounting for approximately 25% of dry weight [12] and at least 19 amino acids have been identified in this plant. Furthermore, Moringa oleifera also contains several minerals [13] and vitamins. The high nutritional content found in dried leaves is an indicator of the usefulness of the plant as a food resource [14][15].

Based on these descriptions, the need for instant liquid food based catfish (*Clarias gariepinus*) and kelor (*Moringa oleifera*) flour are easy to obtain and rich of essential nutrients and other nutrients for toddlers catch up. The objective of this research is to analyze the amino acid composition and protein quality of instant liquid food of catfish flour (*Clarias gariepinus*) and kelor (*Moringa oleifera*) according to the requirement for toddlers.

2. Tools and materials

Tools used in the experiment were Waters Acquity UPLC H-Class and H-Class Bio Amino Acid (AAA) system (Milford, MA, USA), High-Performance Liquid Chromatography (HPLC), sample tube, vortex, analytical weight scale, centrifuge, heating block and volumetric flask. This research also used materials that consisted of HCl, NaOH, alpha-aminobutyric acid (AABA), amino acid standards, AccQ Tag Ultra borate buffer, 1-octanol, sodium citrate buffer, and aqua bidest.

3. Methods

3.1. Amino acid determination

The amino acid composition except tryptophan was analyzed by using Ultra Performance Liquid Chromatography (UPLC). A homogeneous (0.1 g) sample was weighed into sample tube containing 5 mL of 6N hydrochloric acid. The mixture was shaken with vortex for 5 min, then hydrolize at 110°C for 22 hours. Left to rest and transfer to a 50 ml volumetric flask, added aqua bidest to 50 ml. Filter the mixture using 0.45 µm filter papers. Use a micropipette to deliver 500 µl filtrate, 40 µm AABA and 460 µl aqua bidest to a vial and mixed thoroughly. Briefly, for amino acid standard, the 40 µl standard solution was mixed with 40 µl AABA and 920 µl aqua bidest. 70 µL AccQ Tag Ultra borate buffer, 10 mL sample extracts or standard amino acid and 20 µL AccQ Tag Ultra reagent were added to a recovery vial sequentially. The mixed solution was vortexed immediately and allowed to stand at room temperature for 1 min before being placed in a heating block at 55°C for 10 min. After 10 min, the mixed solution was removed, and 1 µL of the sample or standard amino acid was injected into the Acquity UPLC System. All samples were prepared and analyzed in double [16][17].

All analyses were performed on a Waters Acquity UPLC H-Class and H-Class Bio Amino Acid (AAA) system (Milford, MA, USA) equipped with a PhotoDiode Array (PDA) detector, connected to a Waters Empower^{TM} software package for data acquisition and management. A C18 (100 mm × 2.1 mm, 1.7 µm particle size) Acquity UPLC AccQ-Tag Ultra Column also from Waters was used. The standards or samples were separated using a gradient mobile phase consisting of 5% AccQ-Tag Ultra

Eluent A (A) and AccQ_Tag Ultra Eluent B (B). The flow rate was set at 0.70 mL/min and the injection volumes for all samples and standards were 1.0 µL. The column temperature was set at 49°C. The peaks were detected at 260 nm [16][17].

The tryptophan was analyzed by using High-Performance Liquid Chromatography (HPLC). Tryptophan standard solution was prepared by dissolved 25 mg standard L-Tryptophan in 10 ml aqua bidest. Added 0.3 ml HCl and sonicated for 15 min then dilute to 25 ml with aqua bidest. Dilute 1 ml stock solution to 10 ml with aqua bidest and mixed thoroughly. Individual working standard solutions of eight concentration levels were prepared from the stock solution.

Weighed 0.1 g samples into a sealed flask. Deaerated 4.2 M NaOH by bubbling with N_2 for 1 min. Added 10 ml deaerated 4.2M NaOH. Added 3 drop 1-octanol and vortexed immediately. Place flask in the 110°C oven for 20 hours. Let flask cool to room temperature. Quantitatively transfer hydrolysate to 50 ml beaker , rinsing flask with 1 ml portion of 0.2 M pH 4.25 sodium citrate buffer solution three times. Neutralize solution with 3.5 ml HCl and stir vigorously. Adjust pH to 4.25±0.05. Quantitatively transfer solution to 50 ml volumetric flask and dilute to volume with aqua bidest, mixed thoroughly. Pour into a centrifuge tube and centrifuge 20 min at 1150 × g. Filter supernate through glass filter paper then filters the filtrate with 0.45 µm membrane filter GHP. All standard and samples were injected into the HPLC system.

3.2. Protein quality determination

Determination of the protein quality was calculated using the following formula:

$$The\ chemical\ score = \frac{\text{limiting essential amino acid in food protein}}{\text{essential amino acid content in whole cooked egg protein}} \ [18]$$

$$\text{Amino acid score} = \frac{\text{amount of amino acid per test protein [mg/g]}}{\text{amount of amino acid per protein in reference pattern [mg/g]}} \ [18]$$

$$EAAI = \sqrt[n]{\frac{aa_1}{AA_1} x \frac{aa_2}{AA_2} x \dots x \frac{aa_n}{AA_n}};$$

Where: EAAI = Essential amino acid index
aa = A/E ratio in the product, Aa = A/E ratio of 1–3 years old children requirement. [19]

Determination of the total essential amino acid (TEAA) to the total amino acid (TAA), i.e. (TEAA/TAA); total sulphur amino acid (TSAA); percentage cystine in TSAA (%Cys/TSAA); total aromatic amino acid (TArAA), etc.; the Leu/Ile ratios were calculated.

The predicted protein efficiency ratio (P-PER) was determined using one of the equations:
P-PER = -0.468 + 0.454 (Leu) - 0.105 (Tyr) [19].

The predicted biological value (BV) was calculated using the regression equation

$$BV = 10^{2.15} \times q^{0.141}\ Lys \times q^{0.60}\ Phe{+}Tyr \times q^{0.77}\ Met{+}Lys \times q^{2.14}\ Thr \times q^{0.21}\ Trp$$

Where, $q = a_{1\ sample} / a_{1\ reference}$ for a_1 sample $\leq a_1$ reference or
$q = a_{1\ reference} / a_{1\ sample}$ for a_1 reference $\leq a_1$ sample
a_1 = mg of the amino acid per g of total essential amino acid [18]

4. Result and discussion

The protein quality of food depends on the type and amount of amino acids contained in the food [20]. Table 1 showed that the highest essential amino acid content in these instant liquids is the 114.29 mg/g amino acid lysine and the highest non-essential amino acid is the glutamic acid of 244.14 mg/g protein. The lysine content of the sample was higher than the content of the reference egg protein (57 mg/g protein). Catfish flour is thought to be one of the contributors of this type of amino acid in this instant liquid food. Fish and its derivatives contain lots of amino acid lysine compared to other amino acids [21][22]. Arginine (54.50±0.97 mg/g protein) is essential for children and reasonable levels were present here.

Table 1. Amino acid composition and protein quality of instant liquid food.

Essential Amino Acid Type	mg/g protein	Essential Amino Acid Type	mg/gr protein
Lysine (Lys)	114.29±0.79	Glutamic acid (Glu)	244.14±1.86
Leucine (Leu)	112.78±0.76	Aspartic acid (Asp)	109.96±0.49
Phenylalanine (Phe)	70.10±0.70	Proline (Pro)	85.18±0.57
Valine (Val)	68.51±0.25	Serine (Ser)	66.41±0.62
Isoleucine (Ile)	60.02±0.28	Alanine (Ala)	57.91±0.38
Threonine (Thr0	55.94±0.26	Arginine (Arg)	54.50±0.97
Methionine (Met)	30.32±0.05	Tyrosine (Tyr)	43.27±0.28
Tryptophan (Try)	17.11±0.16	Glycine (Gly)	40.89±0.28
Histidine (His)	31.56±0.14	Cystine (Cys)	2.96±0.02

The World Health Organisation recommended Val and Ile requirements for children aged 1-3 years, of 57 and 54 mg amino acid/kg body weight/day [23]. For example, a 15 kg child will require 855 and 810 mg of Val and Ile per day, respectively. The protein values for the instant liquid food (from the proximate composition, g/100 g) were 15.7 g. Consumption of 100 g instant liquid food would provide about 1075 and 942 mg of Val and Ile, respectively. If a 15 kg child, therefore, consumes 100 g of instant liquid food, his FAO/WHO daily requirements of Val and Ile would be met by 126% and 116%, respectively. The same type of calculation holds for the other samples

The contents of TEAA of 529.06±3.25 mg/g protein were close to the value for egg reference protein (566 mg/g protein). The current contents of TEAA are higher to some literature values for freshwater fish, i.e., are M. deliciosus (330 mg/g), B. bayad (395 mg/g), S. budgetti (389 mg/g) and H. fasciatus (394 mg/g) [19]. The contents of TSAA (33.28±0.07 mg/g protein) were generally lower than the 58 mg/g protein recommended for infants [23]. The ArAA range suggested for ideal infant protein (68–118 mg/g protein) [23] has current values meet the requirement. The ArAA are precursors of epinephrine and thyroxin. The percentage ratios of TEAA to the TAA in the sample was 41.80±0.04% which are well above the 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults [23]. The TEAA/TAA percentage contents were strongly comparable to that of egg (50%). Most animal proteins are low in cystine (Cys) and hence in Cys in TSAA. In contrast, many vegetable proteins contain substantially more Cys than Met, for example, 62.9% in coconut endosperm [19]. Although FAO/WHO/UNU [23] did not give any indication of the proportion of TSAA which can be met by Cys in human, information on the agronomic advantages of increasing the concentration of sulphur-containing amino acids in staple foods shows that Cys has positive effects on mineral absorption [24].

Table 2. Summary of amino acid composition and protein quality of instant liquid food.

Protein Quality Variables	Value
Total Sulphuric Amino Acid (TSAA)	33.28±0.07
Total Aromatic Amino Acid (TArAA)	113.36
Total Amino Acid (TAA)	1265.83±8.86
Total Essential Amino Acid (TEAA)	529.06±3.25
%TEAA/TAA	41.80±0.04
%Cys/TSAA	8.90±0.04
%Leu/Ile ratio	187.90±0.32
P-BV	48.51±0.13
P-PER	4.63±0.02
Chemical score	78.30 (Sulphuric Amino Acid)
Amino Acid Score	100
Essential Amino Acid Index	0.98

Adedeye [19] suggested that the leucine/isoleucine balance is more important than a dietary excess of leucine alone in regulating the metabolism of tryptophan and niacin and hence the disease process. The Leu/Ile ratio of instant liquid food was low in value. The predicted biological value of instant liquid food was lower than whey protein (104), egg (100), milk (91), beef (80) and soy protein (74) [3]. Food with a high biological value correlates to a high supply of the essential amino acid. However, the biological value does not take into consideration the influence protein digestibility and interaction with other foods before absorption. The predicted PER value was higher than an egg (3.9), whey protein (3.2), milk (2.5) and soy protein (2.2) [3]. Any PER value that exceeds 2.7 is considered to be an excellent protein source. However, these value (P-PER and P-BV) still need the in vivo evaluation to analyze the PER and BV.

Eggs are an inexpensive and highly nutritious food providing excellent quality of protein [25] with chemical score 100, the highest chemical score of protein source, so eggs serve as reference proteins. Table 2 showed that the chemical scores of the sulphuric amino acid (methionine and cystine) are the lowest so the sulphuric amino acid is a limiting amino acid in this product. Calculation of amino acid score is used to analyze the contribution of essential amino acids in a protein source food to protein requirement for a human. The scores range from 0-100. An amino acid score of the instant liquid food exceeds 100. It indicates that the amino acid scores are very high.

The value of the essential amino acid index (EAAI) was able to predict more precisely the protein quality than the chemical scores and amino acid scores but could not determine the limiting essential amino acid [26][27]. Penafloride [26] stated that good quality protein has an EAAI value of 0.90, useful when around 0.80 and inadequate when below 0.70. Based on the categorization, instant liquid food is a good quality protein source for children aged 1–3 years.

5. Conclusion

This instant liquid food based catfish and moringa flour containing high-quality protein and calcium that is recommended for children aged 1–3 years. Essential amino acid ingredients also make instant liquid food an ideal component of healthy food for children growth and development**.**

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