

Amino Acids Profile and Protein Digestibility in Mocaf's Bread Enriched with High Protein from Local Food

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Amino Acids Profile and Protein Digestibility in Mocaf's Bread Enriched with High Protein from Local Food

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Abstract:- Amino acids profile and protein digestibility in Mocaf's bread enriched with high protein from local food were carried out in Food Technology Laboratory University of Mataram using Completely Randomized Design. Mocaf (modified cassava flour) and tempeh flour formulations are as follows with triplicate : MWTB0 (Wheat 85%: 15% tempeh flour); MWTB1 (Mocaf 85%: 15% tempeh flour); MWTB2 (Mocaf 30%: 55% wheat : 15% tempeh flour); MWTB3 (Mocaf 40%: 45% wheat flour: 15 % tempeh flour); MWTB4 (Mocaf 50%: 35% wheat flour: 15% tempeh flour); MWTB5 (Mocaf 60%: 25% wheat flour: 15 % tempeh flour) and MWTB6 = (Mocaf 70%: 15% wheat flour: 15% tempeh flour). The purpose of this study is to determine the protein digestibility and amino acids profile on mocaf's bread enriched with high protein flour from local food. Protein digestibility and amino acid profile were analyzed. The results showed that the formulation of MWTB6 (mocaf 70%: wheat flour 15%: 15% tempeh flour) produced the highest protein digestibility of 66.65%. MWTB has a composition of 14 essential amino acids and non-essential. The composition of amino acids of MWTB with addition of 15% tempeh flour is predominantly glutamic acid and aspartic acid.

Keywords:- Mocaf Bread, Protein Digestibility, Amino Acids.

I. INTRODUCTION

Indonesia has variety of and cultural resources that need to be preserved its existence, including the localized food. According to the Law of the Republic of Indonesia Number 41/ 2009, local food is both primary and secondary foodstuff produced in an area for economic and consumption purposes. According to the Ministry of Agriculture Food Security Agency, localized food types and amounts and scattered throughout the area that is already well developed is tempeh. Tempeh is one of well known of localized food among the public and has been consumed for generations. In general tempeh made from soybeans that have high proteins content and nutritional value. Judging from its amino acid composition, protein derived from soybeans approaching animal protein. If in a shortage of animal protein diet, it can be replaced by soy protein [7].

Amino acids are organic compounds containing amino and acid groups. Amino acids are the main components that make up the building blocks of protein polymer straight chain by peptide bonds so that the polymer is called a peptide or polypeptide. There are more than 100 kinds of amino acids, but only 20 of these to be used for building blocks of protein [18].

Amino acids are divided into three types of essential amino acids, non-essential, and conditionally. Amino acids that cannot be biosynthesized body called the essential amino acids and needs from food, while the non-essential amino acid is an amino acid that can be synthesized by the body. Conditional amino acids are an amino acid that is usually not essential except in certain circumstances such as illness or stress. The biological function very much like amino acids as the main constituent of the protein component, for growth, body maintenance, energy reserves, and some amino acids act as neurotransmitters. In addition, amino acids make up the basic framework of a number of important compounds in the metabolism (mainly vitamins, hormones, and nucleic acids), as well as metal ion binding with necessary in enzymatic reactions [4].

Tempeh has several advantages compared to soybeans. There are digestive enzymes produced by fungi during tempeh fermentation process, therefore, protein, fat and carbohydrates digestible easily. Mold that grows on tempeh produces protease enzymes to break down proteins into peptides and free amino acids [2].

Soybean composition is very complete amino acids consisting of 15 amino acids with amino acid content varied from 0.04 to 0.56% range. Tempeh contains large amounts of glutamic acid at 0.4% which could be assist iron absorption with Protein Efficiency Ratio (PER) is similar to cow's milk casein [14]. However, it has a relatively short shelf life product due to of the growth of bacteria or microbial degradable protein thus becomes decomposed tempeh [17].

Tempeh processing into flour has many advantages such as easy to store, add shelf life, easily mixed with other nutrients in order to improve the nutritional value, and can be processed into more variety of food. According Mardiah

[13], the nutritional value of soybean flour is very high, reaching 48% protein. According to Mahmud *et al.* [12], the value of digestibility, biological value and Net Protein Utilization (NPU) tempeh flour is 91%, 92%, and 84% respectively. Manufacture of complementary feeding applies it as porridge and biscuits. In addition, tempeh flour also been developed in the manufacture of bakery products such as bread and cake. According to Saloko *et al.* [16], the addition of tempeh flour to produce bread by 15%, which has a high protein content and acceptable by panelists. In addition tempeh flour is also could produced brownies that have the highest protein content [22]. Although research on the use of soybean flour in making bread, especially mocaf's bread already complete but has not been fully explored on the amino acid profile and its digestibility. **The purpose of this study is to determine the protein digestibility and amino acids profile on mocaf's bread enriched with high protein flour from local food.**

II. MATERIAL AND METHODS

This observation was carried out from June to October in the Laboratory of Food Processing, Laboratory of Food Chemistry and Biochemistry, Faculty of Food Technology and Agro-Industry University of Mataram, and the Organic Chemistry Laboratory of the Faculty of Science University of Gadjah Mada. Experimental design in laboratory using Completely Randomized Design (CRD) with mocaf substitution and tempeh flour treatments are as follows with triplicate : MWTB0 (Wheat 85%: 15% tempeh flour); MWTB1 (Mocaf 85%: 15% tempeh flour); MWTB2 (Mocaf 30%: 55% wheat : 15% tempeh flour); MWTB3 (Mocaf 40%: 45% wheat flour: 15 % tempeh flour); MWTB4 (Mocaf 50%: 35% wheat flour: 15% tempeh flour); MWTB5 (Mocaf 60%: 25% wheat flour: 15 %tempeh flour) and MWTB6 = (Mocaf 70%: 15% wheat flour: 15% tempeh flour).

Observation protein digestibility trials were analyzed descriptively by standard deviation using Microsoft Excel Software. While the amino acids data were analyzed descriptively and presented into narrative and tables. The process of making bread mocaf's bread enriched with high-protein from local food can be seen in Figures 1.

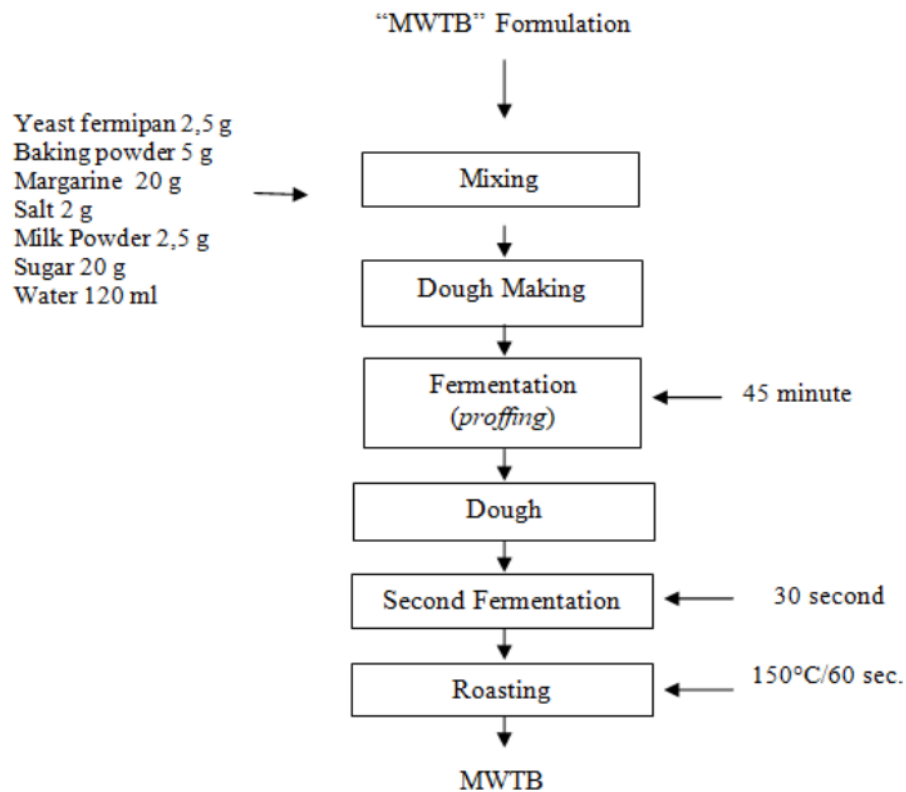


Fig 1:- Flow chart of Mocaf's bread enriched with high protein from local food.

➤ *Protein Digestibility [19]*

A sample of 5 g into Erlenmeyer flask and added with 20 ml Buffer pH Whaffole 2 of 20 ml. Then added 2 ml of the pepsin enzyme 1% and incubated at 40 °C for 1 hour. Filtered or centrifuged the solution and added 5 ml of 5% TCA. Allowed to stand for 1 hour and then take 5 ml filtrate for analysis of protein content.

Protein Digestibility (%) =
 (% enzyme protein content) / (% total protein content) x 100%

➤ *Amino Acids Analysis [1]*

“RMTP” Amino Acids analysis by HPLC (Shimadzu LC 10A) was performed with a system consisting of Water 470 pump detector. Sample preparation is done by weighing 60 mg of sample + 4 ml of HCl 6 N reflux for 24 hours at 110°C. Cooled and neutralized with NaOH to pH 7 and filtered with Whatman 0.2 μm. Take the 50 μL sample was added OPA 300 μL and injected into the HPLC as much as 20 μL. Samples were injected using a Waters U6K injector onto Column LiChrospher 100 RP-18 (5μm). detector (Waters 470), Solvent A: 50 mM Na-acetate and Na-dibasicfosfat: THF: Methanol (96 : 2 2), Solvent B: 65% methanol. Samples were eluted from the column at a flow rate of 1.5 mL.min⁻¹ with a programmed solvent gradient of 0.1, 100, 0; 2, 100, 0; 35, 0, 100; where the first number was the time (minutes), the second number was the percent of Solvent A (50 mM Na-acetate and Na-dibasicfosfat: THF: Methanol (96 : 2 2), and the third number was the Solvent B (65% methanol). The flow rate of water: 1.5 ml / min. Elution was completed in 40 min. Products were detected with a Tunable Absorbance Detector (Waters 470) using an excitation wavelength of 365 nm.

III. RESULTS AND DISCUSSION

➤ *Mocaf Bread*

Mocaf's bread (MWTB) products resulting from this study are presented in Figure 3. Mocaf's bread enriched with high protein from local foods such as tempeh flour gives the result that the higher the addition of mocaf flour visually shows the smaller bread volume. Bread volume buns development has additional size before and after baking. Standard of bread volume which is twice of the volume of the initial dough [21].

The high-protein bread enriched mocaf local foods such as tempeh flour gives the result that the higher the addition of mocaf flour visually show smaller level of enlargement. Bread volume is the ability bread buns development of size before and after baking process. Standard volume development of bread which is twice the volume of the initial dough [21].

Bread in the treatment of additional flour only (MWTB₀) provides bread volume better than other treatments that use mocaf. This is due to mocaf and tempeh flour used for the formulation did not have a gluten content so that both viscosities are lower than that of wheat flour. Gluten preserves gas to obtain the desired volume and texture in dough systems. Gluten consists of two fractions, namely glutenin and prolamin where prolamin provide viscosity and dough extensibility, while glutenin function provides the elastic properties and cohesive dough [11]. In addition, the high protein in the treatment MWTB₀ bread produced resulting in darker color because of the high protein tends to be easily oxidized due to the heat and the Maillard reaction [23].

➤ *Digestibility of Protein of Mocaf's Bread*

Mocaf's bread protein digestibility of high-protein in various formulations of treatment is presented in Table 1. In general, the levels of protein in food ingredients determine the quality of food itself. The amount of protein consumed, among others, determined by the nutritional value of protein. The nutritional value of proteins is influenced by two factors such as composition and digestibility of amino acids essential constituent.

The ability of a protein to be hydrolyzed into amino acids by digestive enzymes (protease) is known as protein digestibility. Protein has a high digestibility, the amino acids that can be utilized by the body to be high. Preferably proteins that stodgy, then most will be excreted through the feces.

Based on descriptive analysis can be seen that mocaf bread with the addition of mocaf flour tended to give higher protein digestibility (Table 1). The level of protein digestibility differences resulting from the effect of adding of mocaf despite the addition of tempeh flour given the same proportion of the number is 15%. It shows more and more additions mocaf flour causes increased protein digestibility. Protein digestibility of bread produced by 33.38% - 66.65% with MWTB₁ have the lowest protein digestibility and MWTB₆ has the highest protein digestibility.

Formulations	Protein Digestibility (%)			Average
	I	II	III	
MWTB ₀ Flour 85% : tempeh flour 15%	54,54	54,89	54,67	54,70
MWTB ₁ Mocaf 85% : tempeh flour 15%;	33,10	33,62	33,43	33,38
MWTB ₂ Mocaf 30% : Flour 55% : tempeh flour 15%;	43,04	43,32	43,22	43,19
MWTB ₃ Mocaf 40% : Flour 45% : tempeh flour 15%;	49,72	50,03	49,89	49,98
MWTB ₄ Mocaf 50% : Flour 35% : tempeh flour 15%;	51,91	52,14	52,01	52,02
MWTB ₅ Mocaf 60% : Flour 25% : tempeh flour 15%	55,94	56,75	56,25	56,31
MWTB ₆ Mocaf 70% : Flour 15% : tempeh flour 15%.	69,59	68,52	68,74	66,65

Table 1:- Digestibility of Mocaf's bread enriched with high protein from local food in various formulations.

An easily digested protein showed that high number of amino acids that can be absorbed and used by the body. Conversely, most protein that is difficult to digest will be discarded by the body. According to Suhardjo and Kusharto [21], the digestibility of the protein begins with an overhaul of the peptide bond that occurs in the stomach by using an acidic medium of gastric juices. Gastric fluid produces "protein-splitting enzyme" that pepsin (gastric protease) working remodel peptide bonds of proteins into amino acids shorter called peptone. From the stomach, the protein digested will go into the intestine, where the media have been neutralized acids become slightly alkaline [9]. Fluid pancreas produces two kinds of "protein-splitting enzyme" is an enzyme trypsin and chymotrypsin (pancreatic proteases), and 30% of the proteins reorganized into simple amino acids and directly absorbed by the intestine, while 70% of the protein is broken down into a dipeptide, tripeptide, or comprises more of three amino acids. In accordance with Wu *et al.* [24] indicated that digestibility generally increased during proofing and decreased during baking. Relatively higher protein digestibility was correlated with ratio of non-fibre carbohydrate to protein and lower digestibility with increasing contents of fibre and total polyphenolics in pre-mixes.

➤ Amino Acid profile Mocaf's Bread enriched with High Protein

The observation of the amino acid profile of high-protein amino mocaf's bread in all formulations of treatments showed that all samples containing essential amino acids and non-essential as shown in Table 2.

Amino acids content in MWTB₀ ranged from 0.0592 to 0.7067 ppm, with the dominant of glutamic acid. The glutamic acid value is also the most dominant for all formulations of the treatment, and tends to decrease with the increasing addition of mocaf. High content of Glutamic acid on the food product might support the absorption of iron.

It can be seen that from the composition of amino acids, vegetable protein in soybeans have amino acid sequence that is approaching the animal protein. Protein from tempeh is made from raw soybeans including proteins that have high nutritional value, with amino acid sequence that is closer to the component of animal protein in a diet so that when there is a shortage of animal protein, it can be replaced by protein from the tempeh.

Amino acids	Amino Acids (ppm)+						
	MWTB ₀	MWTB ₁	MWTB ₂	MWTB ₃	MWTB ₄	MWTB ₅	MWTB ₆
Aspartate	0,2146	0,1455	0,1673	0,1424	0,1485	0,1320	0,1370
Glutamate	0,7067	0,2195	0,4683	0,3728	0,3740	0,3039	0,2676
Serin	0,1312	0,0659	0,0980	0,0831	0,0846	0,0707	0,0686
Histidine	0,0592	0,0308	0,0420	0,0351	0,0354	0,0371	0,0284
Glysin	0,0849	0,0340	0,0552	0,0481	0,0489	0,0414	0,0119
Threonine	0,0773	0,0404	0,0598	0,0501	0,0522	0,0443	0,0365
Arginine	0,1190	0,0503	0,0867	0,0763	0,0765	0,0688	0,0777
Alanine	0,1143	0,0671	0,0857	0,0750	0,0751	0,0680	0,0667
Tyrosin	0,1303	0,0615	0,0925	0,0772	0,0786	0,0564	0,0540
Valine	0,0937	0,0406	0,0638	0,0503	0,0525	0,0448	0,0420
Phenil-alanine	0,1124	0,0497	0,0807	0,0621	0,0656	0,0558	0,0501
Isoleucine	0,0901	0,0413	0,0616	0,0506	0,0547	0,0468	0,0414
Leucine	0,1806	0,0909	0,1350	0,1103	0,1135	0,0951	0,0927
Lysine	0,0882	0,0554	0,0590	0,0637	0,0578	0,0489	0,0477

Table 2:- Amino acids composition of Mocaf's bread enriched with high protein

+) MWTB₀ = Flour 85% : tempeh flour 15%; MWTB₁ = Mocaf 85% : tempeh flour 15%; MWTB₂ = Mocaf 30% : Flour 55% : tempeh flour 15%; MWTB₃ = Mocaf 40% : Flour 45% : tempeh flour 15%; MWTB₄ = Mocaf 50% : Flour 35% : tempeh flour 15%; MWTB₅ = Mocaf 60% : Flour 25% : tempeh flour 15%; MWTB₆ = Mocaf 70% : Flour 15% : tempeh flour 15%.

Amino acids identified in all formulations classified as an essential amino acid is complete, namely lysine, isoleucine, leucine, threonine, phenylalanine, valine, histidine and arginine. Similar amino acids (Asp, Ala, Phe, Leu, Val) also found in bread and toast [5,9]. According to Winarno [23], arginine is not essential for children and adults but is useful for the growth of the baby, while histidine is essential for children but it is not essential for adults. Thus the amino acids arginine and histidine is essential for infants and children [8]. Nittynen *et al* [15] suggests that the amino acid arginine is able to give the effect of a reduction in "atherogenesis" associated with heart and blood vessel disease. And also mentioned the effect of supplementation with the amino acid arginine and other aspects of the human physiology are not yet clear. Deliani [7] states that the fermented soy can cause the food are easily digested and unpleasant odor of soybeans will be lost. Fermentation can also affect the value / composition of amino acids contained in the food [6,10]. Amino acid composition is meant is the value of tryptophan formed during 0-24 hours of fermentation after that decrease, and then the amino acids methionine and lysine will be lost during the fermentation 4-115% methionine and 10-24% lysine respectively. Amino acids are usually very lacking in foodstuffs referred to as the limiting amino acid. The limiting amino acid is lysine, while in group of legumes (beans) are usually amino acid methionine [23,4,3]. When the two types of essential amino acids consumed together then the shortage of some amino acids would be able to equalize by the excess amino acids from another protein. Two of these proteins mutual support and improve the quality of nutrition in mixed conditions than standing alone. Furthermore, Winarno [23] stated that the biological value of food can escalate if made the right mix and this occurrence unnoticed by humans every day. The mixture can be either milk with cereal, rice with tempeh, beans with bread or meat. All the mixture is a combination of menus that can improve the quality of protein [4].

IV. CONCLUSION

Formulation treatment of wheat flour: mocaf flour: tempeh flour produces a tendency towards an increase in the digestibility of protein mocaf bread with the increasing addition of mocaf flour. MWTB₆ treatment mocaf bread (mocaf flour 70%: wheat flour 15%: 15% soybean flour) produced the product with the highest protein digestibility of 66.65%. Mocaf bread has the variety of amino acids, there are 14 essential amino acids and non-essential. The amino

acid composition is predominantly glutamic acid and aspartic acid with addition of 15% tempeh flour in mocaf's bread. Bakery products mocaf need formulations that higher in tempeh flour, in order to gain the bread contain amino acids and high protein digestibility.

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