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Amino Acid Characterization of Tofu Waste Fermentation using Effective Microorganism-4 and *Lactobacillus plantarum* Culture

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Abstract

The research aimed to know the amino acid characterization of tofu waste fermentation using effective microorgansism-4 (EM-4) and *Lactobacillus plantarum* culture with different concentrations. Research used Random Nested Design with 2 factors, factor 1 was a type of microbe (EM-4 and *Lactobacillus plantarum)* and factor 2 was the concentration of microbes consists of five levels (1%, 5%, 10% , 15%, and 20%, v/w). The results showed that the treatments giving very significant effect on amino acid parameter. The 20% concentration of *Lactobacillus plantarum* treatment producing 1612.72 mg/100 gr valine, 587.44 mg/100 gr methionine, 1694.62 mg/100 gr isoleucine, 2123.93 mg/100 gr leucine, 820.90 mg/100 gr tyrosine, 1468.8 mg/100 gr phenylalanine, 832.94 mg/100 gr histidine, 1632.66 mg/100 gr lysine, 1858.79 mg/100 gr arginine, 607.05 mg/100 gr tryptophan, 566.84 mg/100 gr glutamine, and 545.78 mg/100 gr cystine, higher than EM4. Methionine and lysine are limiting amino acid, which is crucial for animal growth.

Keyword : *amino acid, fermentation, tofu waste, EM-4, Lactobacillus plantarum*

Introduction

Amino acids are essential nutrients and should be in the food. In some ways amino acids can substitute other materials as a source of protein, helps in the storage protein or as a protein reserves, and reduce nitrogen excretion, and indirectly lower the cost of feed. As an example, lysine is resulting in the protein deposition and muscle growth, and it also has other functions such as improving digestion. Threonine in the diet can improve immunity (Anonymous, 2016). Increased content of lysine in the diet can increase the body weight of broilers (Anonymous, 2016) and resulted in the production of breast meat in hight result (Barboza *et al*., 2000). Threonine not only play a role in metabolism, but also involved in the maintenance process, such as updating the intestinal mucus and the synthesis of proteins associated with immune. Threonine is the third limiting amino acid after lysine and methionine.

Amino acids are chemically bound to the protein and should be broke from the main protein. This process occurs in the intestine with the aid of proteolytic enzymes. Amino acids and oligopeptides are absorbed in the intestinal wall and enter the bloodstream as free amino acids (Dalibard *et al*., 2014). Today the use of amino acids as an additive in feed is crucial and must be added, either to feed poultry and ruminants. One source of protein that is widely used as animal feed is tofu waste. But it has some problems due to their anti-nutrients and amino acid content is low.

The using microbial fermentation is needed to solve the lack of nutrients in tofu waste. In this study using microbes EM4 and *Lactobacillus plantarum*. Tofu waste is a high protein

feed source and is rich in isoflavones (Astuti and Noviana, 2013). The use of two groups of microbes is expected to increase the amino acid content of fermented tofu waste.

Methodology

The research was done in Engineering Laboratory of Food in Tribhuwana Tunggadewi University, East Java. *Lactobacillus plantarum* was obtained from the Laboratory of Agricultural Product Technology, Brawijaya University, Indonesia. EM4 is effective microorganism-4 of PT Persada Songgolangit product which containing microbes *Lactobacillus casei* 1. $5x10^6$ cfu/ml, *Saccharomyces cerevisiae* 1 .$5x10^6$ cfu/ml, and *Rhodopseudomonas palustris* $1.0x10^6$ cfu / ml. Amino acid analysis was done in biochemical laboratory University of Muhammadiyah Malang.

Culture of *Lactobacillus plantarum* was made by boiling 200 g of potatoes (it was peeled and cut into small size) in 1000 ml of distilled water. Potato extract solution is filtered into Erlenmeyer, added by 15 g of sugar, stirring until dissolved. Before inoculated using *Lactobacillus plantarum*, all equipment and media were sterilized using an autoclave. On other hand, EM4 pure solution was diluted using distilled water. As an example for the needs of 1000 g tofu waste with 1% concentration EM4, the EM4 pure solution was diluted, its calculation was 1/100 x 10 ml = 0.1 ml. Next, 0.1 ml of EM4 inserted into a measuring cup and add 10 ml of distilled water until reaching 10 ml, then newly mixed into the tofu waste.

The Research used Random Design Nested two factors, the treatment consists of:. Factors I: Microbial type, consisting of two levels, namely M1 = *Lactobacillus plantarum*, M2 = Effective Microorganism (EM-4); Factor II. The concentration of microbes, consisting of five levels, namely K1 = 1%, K2 = 5%, K3 = 10%, K4 = 15% K5 = 20% (v/w) where the second factor is nested on the first factor (microbes) to 3 replications

Research Activities

Tofu waste was squeezed to reducing the water content, steamed for 30 minutes and cooled. The sugar as much as 1% and 5% skim milk was added into cooled tofu waste then fermentation was performed using two groups of microbes in accordance with the treatment. Fermented tofu wate performed in an airtight plastic and fermented for 4 days. Fermented tofu waste opened and dried in an oven temperature of 60 °C, set it to dry analysis of amino acids. Determination of amino acid analysis using HPLC.

Result and Disscusion

. Tofu is a food made from freshly precipitated soy beans which undergo coagulation caused by the addition of vinegar in the manufacturing process. Tofu has a moisture content 84.9-87.3%, 6.5-8.8% protein, 4.1-4.6% oil (Wang and Calvins, 1989). Tofu waste is a waste processing from tofu. The protein content of each product will vary depending on the materials used soybean and comparison with the water content. Data of fermented tofu waste comparing between two groups of microbes, EM4 (mixing bacteria consisting of *Lactobacillus casei, Saccharomyces cerevisiae*, and *Rhodopseudomonas palustris*) and *Lactobacillus plantarum* (single bacteria), is shown in Table 1. Compared with unfermented tofu waste, the result of fermented treatments, amino acids content of the average increases in both groups of microbes. However,. The results showed that *Lactobacillus plantarum* treatments give higher yields to all 14 amino acids compared to microbial EM-4.

Korhonen and Pihlanto (2003) said during microbes activity at fermentation process, it can release free amino acids and bioactive peptides. The research result is in line with research and Cavins Wang (1989), that the manufacturing process of tofu causes an increase in the amino acid valine, methionine, isoleucine, leucine, tyrosine, and phenilalanine. In this study, tofu waste is getting fermentation again using two groups of microbes and the results

showed an increase in all of the amino acids. Amadou *et al*. (2010) reported that fermentation of soybean by *Lactobacillus plantarum* release amino acids and forming peptides which have low molecular weights. Compared with the control (without fermented soybeans), fermented causing increasing amino acid valine, isoleucine, and leucine.

Table 1. Data influence of fermented tofu by EM4 and *Lactobacillus plantarum* to the amino acid content

Treatment	val	met	ile	leu	tyr	phe	his	Lys	arg	trp	asp	gln	cys
	(mg/100 g)												
Tofu waste (non fermented)	1246.1	403.0	1312.7	1666.8	639.9	1126.9	672.8	1329.5	1532.7	470.1	300.9	386.5	369.3
M1K1	1322,8a	440,3a	1393,4a	1763,3a	713.6	1198,4a	651,4a	1338,5a	1531.9	457,7a	280,5a	422,1a	404,1a
M1K2	1422,4a	490,1a	1497,1a	1888,1a	750.7	1292,7a	713,7a	1441,6a	1646.6	507,7a	303,8a	471,1a	452,6a
M1K3	1424,6a	491,2a	1499,6a	1891,9a	752.6	1293,1a	715,0a	1443,6a	1647.3	509,3a	303,7a	471,8a	453,4a
M1K4	1450,1b	503,8b	1525,6b	1922,1b	768.5	1316,9b	730,6b	1467,9b	1674.8	523,1b	314,2a	485,1b	466,1b
M1K5	1531,2b	545,0a	1609,8b	2024,2c	820.9	1393,0b	781,2b	1551,3b	1767.2	565,1b	346,7b	525,7b	505,3b
M2K1	1323,8a	440,5a	1395,3a	1764,8a	687.5	1199,2a	652,9a	1341,1a	1535.5	457,2a	281,4a	422,5b	404,3a
M2K2	1375,7a	466,1a	1448,1a	1828,7a	720.7	1245,1a	684,3a	1393,0a	1592.0	484,5a	284,3a	448,1a	429,7a
M2K3	1395,9a	479,0a	1473,8a	1860,5a	736.3	1270,9a	699,6a	1417,9a	1621.8	497,1a	294,4a	460,2a	441,5a
M2K4	1503,2b	530,9b	1581,2b	1987,8b	800.9	1366,1b	763,9b	1522,0b	1735.8	549,7b	334,9b	511,4b	491,4b
M2K5	1612,7c	587,4c	1694,6c	2123,9c	748.9	1468,8c	832,9c	1632,6c	1858.7	607,0c	312,4a	566,8c	545,7c
BNT	P<0.01	P<0.01	P<0.01	P<0.01	p>0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	p>0.05	P<0.01	P<0.01

Conclusion

The results showed that the treatments giving very significant effect on amino acid parameter. The 20% concentration of *Lactobacillus plantarum* treatment producing 1612.72 mg/100 gr valine, 587.44 mg/100 gr methionine, 1694.62 mg/100 gr isoleucine, 2123.93 mg/100 gr leucine, 820.90 mg/100 gr tyrosine, 1468.8 mg/100 gr phenylalanine, 832.94 mg/100 gr histidine, 1632.66 mg/100 gr lysine, 1858.79 mg/100 gr arginine, 607.05 mg/100 gr tryptophan, 566.84 mg/100 gr glutamine, and 545.78 mg/100 gr cystine, higher than EM4

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PAGE 1

PAGE 2

PAGE 3