

Short Communication

The Difference in *Lactobacillus plantarum* Density on the Fermentation Process of Cassava Leaves (*Manihot utilissima*) as Substance for Plant-Based Protein

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Abstract

Cassava leaves (*M. utilissima*) are well used as a staple ingredient for feeding livestock mainly for high nutrient content especially protein which is 27%. The main problem in the use of cassava leaves as fish feed ingredients is its high fiber content, making it difficult to be digested by fish. The main purpose of this research was that fermented cassava leaves by using *L. plantarum* could increase its protein content. This research was conducted by adding the different concentrations of *L. plantarum* which were 0 cell/ml, 10^{12} cells/ml, and 10^{13} cells/ml, 10^{14} cells/ml, and repeated three times. The result showed that *L. plantarum* with the concentration of 10^{14} cells/ml provides the best results overall in increasing protein content by 35.8% and physical characteristic test including scent, texture, hypha quantity, and water vapor provides the best result.

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1. Introduction

Cassava leaves (*Manihot utilissima*) are one of main ingredient for feed alternatives that has maximum nutritional value, and valuable for fish farming especially to increase digestive enzyme activity, nutrient efficiency, growth, economical, and abundant to be obtained. Main ingredients are categorized as protein sources if it contains more than 18%. While cassava leaves has raw protein content of 27% which is suitable for carnivorous and herbivorous fish. Cassava leaves has amino acid content including lysine (2.00%) and methionine (0.40%). Ratio of amino acid lysine to protein in cassava leaves flour are (7.4%) which is equal to the value on fish flour (7.7%) and higher than soybean meal (6.30%) (Askar, 2006). Cassava leaves (*M. utilissima*) are very good for use as raw material for human food and animal feed because cassava leaves contain very high levels of vitamin A, vitamin C, minerals (Fe, Ca), and protein (OECD, 2009). Hence, cassava leaves can be one of protein sources in fish feed and capable of alternative sources for livestock staple food. Unfortunately, the fiber in cassava leaves are too complex to be digested by fish and its HCN (anti nutrition) content can interfere the growth of fish so that it needs to be break down to be able to digest by fish in cultivation. This substance is a covalent molecular substance that can dissociate in water, and is a very poisonous and colorless gas. This type of substance is a strong poison that will cause asphyxics (hypoxia) and hinder with oxidation (transport of O₂) to body systems by binding to oxidative cytochrome enzymes. This bond cannot be used by the tissue so that organs that require more oxygen will be damaged, especially brain system which can be seen from the central nervous system which will be followed by stress levels that lead to seizures by hypoxia to death caused by respiratory problems (DHHS, 2006). The dosage that can be lethal in the use of cyanide is 3 mg/kg. As for how to improve the nutritional quality of an ingredient so that it is easily absorbed and digested by fish is by carrying out a fermentation process that utilizes microorganisms as a medium during the fermentation process. Then, to reduce the anti nutrition in cassava leaves, it need to carrying out boiling process before it used to main ingredient of fish feed (Rawat, 2015).

One of the microorganisms that is often used in the fermentation process is the type of *L. plantarum*. This type of bacteria is gram positive, facultative anaerobic rods. This *Lactobacillus* bacteria is a genus of a group of lactic acid bacteria (LAB) that live in the digestive

tract and play a role in the process of food fermentation (Puspadewi et al., 2011). One of the important in the application of lactic acid bacteria for fermentation process is the ability of these bacteria to produce bacteriocin to inhibit the growth of various pathogenic bacteria (Aliya et al., 2016). It has antagonistic properties against microorganisms that cause food degradation such as *Salmonella*, *Staphylococcus aureus*, Gram negative. These bacteria are salt tolerant bacteria, that can produce acid quickly and have an ultimate pH of 5.3 to 5.6 (Buckle et al., 1985). Bacteriocin produced by lactic acid bacteria functions as an anti bacterial, it will make balance in fish digestibility so that the digestive tract of fish becomes better at digesting and absorbing nutritious food (Purba, 2017). The feed that use *Lactobacillus* bacteria can increase growth, immune response, and disease resistance in the *Ephinephelus coioides*, *Ephinephelus bruneus*, and tilapia (*Oreochromis niloticus*) groups (Abumourad et al., 2013). This research's main focus is to give solution for fish farmer to utilize the cassava leaves as main ingredient in alternative fish feed which has high protein to increase fish growth.

2. Materials and Methods

2.1 Cassava Leaves Fermentation

Cassava leaves are cleansed by using running water. Boiling is carried out to reduce levels of anti-nutrient substances in cassava leaves. After boiling there was reduction in the anti nutritional composition such as nitrate and tannin (Lola, 2009). Next is to dry the leaves under sunlight two days and for 7 hours. Dried cassava leaves are mashed using a diskmill, sifted then weighed and put into plastic. Flour that has been added to the plastic then added *L. plantarum* bacteria as much as 1.5% in the leaves to be fermented (Dewi et al., 2010) by being given a density treatment that is 10¹² cells/ml, 10¹³ cells/ml, 10¹⁴ cells/ml. The bacteria counted by Optical Density (OD) and Total Plate Count (TPC) methods. It used to the different dilution in the test tube so it could be decided the density of 10¹ cell/ml until 10¹⁴ cells/ml Then bacteria was planted in Nutrient Agar (NA) during a day, and counted the density level by regression score. Then added molase as much as 2,5 kg/100 kg and put in a closed box in an anaerobic state. The process are checked on the 7th day, 14th day, and 21st day.

2.2 Physical Characterization

The method was used in the study physical characteristic organoleptic which made on the fermentation process are changes in texture, aroma,

amount of hyphae, amount of water vapor on the surface of fermented cassava leaves (Sukardi *et al.*, 2008) then made in the form of scoring to show the success of the fermentation process at each treatment with the density of *L. plantarum* used which were 0 cell/ml, 10^{12} cells/ml, 10^{13} cells/ml, 10^{14} cells/ml. Score range between 1-4 to determine quality where the higher the score implies better fermentation.

2.3 Proximate Analysis

Proximate analysis was carried out on cassava leaves before and after fermentation with the density of *L. plantarum* used, namely 0 cell/ml, 10^{12} cells/ml, 10^{13} cells/ml, 10^{14} cells/ml. Proximate analysis is carried including protein, fat, water, and ash contents, crude fiber, and BETN.

2.4 Statistical Analysis

The data was analyzed by using the SPSS version 16.0 program with one way ANOVA test. The analysis is used to test the effect of treatment, followed by using the least significant difference test (LSD) with the Duncan test method. From this test continued with orthogonal polynomial analysis to determine the response test (AOAC, 1995).

3. Results and Discussion

Cassava leaves fermentation scoring result are shown based on following graphics on Figure 1. From the scoring graph on Figure 1 obtained the best *L. plantarum* dose and fermentation time is density of 10^{14} cells/ml within 21 days. It is obtained from observations of physical characteristics such as aroma, texture, amount of hyphae, water vapor that observed in the fermentation process. From the observation on 7th day, cassava leaves texture had raw leave surfaces and faded easily. However on 14th day, it had few lumps in 10^{12} cells/ml and 10^{13} cells/ml dose. The most lumps was found in 10^{14} cells/ml dose started on 14th until 21st day.

The lowest of number of Hyphae had result in 10^{14} cells/ml dose. The small amount of hyphae that surrounds the fermentation of cassava leaf is caused by *L. plantarum* having antifungal properties. The antifungal ability produced by LAB produces bioactive compounds produced in the form of hydrogen peroxide, diacetyl, and bacteriocin which work antagonistically against pathogenic, and hyphae bacteria. Antipatogen compounds found in LAB in the form of proteins, proteins produced by *L. plantarum* in the form of *cyclo cyclic dipeptides* which can inhibit mold growth (Strom *et al.*, 2002).

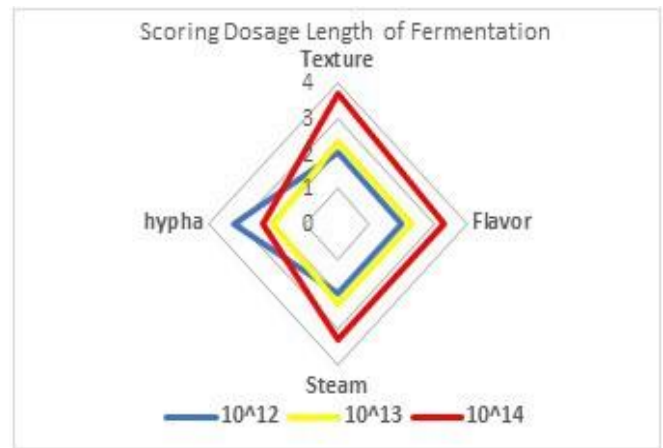


Figure 1. Fermentation duration score result

Fresh and fragrant aromas produced from all treatments with fermentor bacteria are good quality silage characteristics. This is influenced by the fermentation process that produces lactate acid (Zakariah *et al.*, 2015). During the fermentation process, LAB type bacteria produce enzymes in degrade the substrate, one of them enzymatic processes during the fermentation process. Enzymes broke the fat and protein to release a fragrant fermentation. It produced from lactic acid excretion that released organic acid. The substance had released by lactic acid bacteria and volatil component built acid and characteristics aroma (Edam, 2018). The presence of enzymes when lactic acid bacteria in the fermentation process will be able to change the texture, aroma, taste, and increase the nutritional value (Suri *et al.*, 2013). Table 1 shows the scoring percentage in or- ganoleptic process.

The results of cassava leaves that have been fermented with the best fermentation results carried out the density of *L. plantarum* bacteria that is 10^{14} cells/ml within 14 days. The use of fermentation is intended to reduce levels of crude fiber, and improve protein quality. The results can be seen in Table 2. The results of the proximate fermentation analysis presented in Table 2 show that fermentation using *L. plantarum* can increase protein levels in fermented cassava leaves. The highest level of protein testing occurred at the use of 10^{14} cells/ml bacteria which produced a protein content of 35.87%, then followed by the use of 10^{12} cells/ml bacteria (26.23%), and 10^{13} cells/ml (26.96%). While the lowest protein content results are shown in leaves without fermentation using *L. plantarum*. Increased protein levels during the fermentation process using LAB is caused because during the fermentation process LAB will produce peptidoglycan compounds found in cell walls, which are known to be composed of lipoprotein and glycoprotein components (Reddy *et al.*, 2008).

Table 1. Scoring criteria for physical tests on fermentation

Score Value	Status	Number of Lumps	Aroma	% Vapor	Number of Hyphae
1	Very Poor	Soft < 10%	Rancid > 40%	Dry < 10%	None < 10%
2	Poor	Slight Lump < 10 - < 25%	Slight Rancid > 25 - < 40%	Slightly humid < 10 - < 25%	Small < 10 - < 25%
3	Good	Moderate > 25 - < 40%	Slight Fragrant > 10 - < 25%	Moderate Amount > 25 - < 40%	Moderate > 25 - < 40%
4	Excellent	Abundant > 40%	Fragrant < 10%	Abundant > 40%	Abundant > 40%

Table 2. Proximate analysis of cassava leaves fermentation

Bacteria Dosage	Protein (%)	Fat (%)	Water (%)	Ash (%)	Crude Fiber (%)	Carbohydrate (%)
0 cell/ml	24,92±0,57 ^a	6,57 ±0,51 ^a	92,04 ±0,65 ^a	9,77 ±0,61 ^a	26,56 ±0,6 ^a	32,18 ±1,12 ^a
10 ¹² cell/ml	26,96±0,72 ^a	6,52 ±0,66 ^a	92,54 ±0,57 ^a	11,68 ±0,70 ^b	29,21 ±0,54 ^a	25,63 ±1,15 ^a
10 ¹³ cell/ml	26,23 ±0,50 ^a	7,74 ± 0,57 ^a	92,73 ±0,59 ^a	9,72 ±0,59 ^{ab}	22,10 ±0,60 ^a	34,21 ±1,98 ^a
10 ¹⁴ cell/ml	35,87 ±0,74 ^b	5,74 ± 0,33 ^a	91,75 ±0,39 ^a	8,62 ±0,39 ^a	18,33±1,07 ^a	31,44 ±1,07 ^a

The proximate analysis used *L. plantarum* increased protein, fat, carbohydrate, and energy. While the fermentation process, CO₂ and H₂O was released caused the breaked out of organic compound in fermentation media. Fermentation process used to increase the feed digestibility through the breaked out complex organic compound including carbohydrate, fat, protein, and other organic compound (Sahlin, 1999). Gunawan (2015) had studied the fermentation of cassava leaves within different time of fermentation which used lactic acid bacteria was result that increased protein 8% than before fermentation. One of the bacteria that could be used to increased protein to 8.52% is *L. Plantarum*. It was caused by bacteria released some extracellular enzyme such as proteinase.

4. Conclusion

Conclusion from the results showed that the fermentation of cassava leaf flour by using *L. plantarum* bacteria at a density of 10¹⁴ cells/ml gave the best results in increasing the protein content by 35.87% and the physical characteristics test in the form of aroma, texture, amount of hyphae, water vapor gave the best results.

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Conflict of Interest

The authors declare that they have no competing interests.

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