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PENGARUH PENAMBAHAN BERBAGAI MEDIA SUBSTRAT MIKROORGANISME LOKAL (MOL) TERHADAP KANDUNGAN NUTRISI DAN ASAM SIANIDA FERMENTASI ANAEROB KULIT SINGKONG (*MANIHOT UTILISSIMA*)

The Effect of Adding Various Media Substrates of Local Microorganisms (MOL) on The Nutrient and Cyanide Acid Content of Cassava Peels (Manihot utilissima) Anaerobic Fermentation

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ABSTRAK

Keterbatasan hijauan pakan menyebabkan perlunya pakan alternatif untuk memenuhi kebutuhan ternak, salah satu pakan alternatif yang dapat digunakan adalah kulit singkong terfermentasi. Penelitian ini bertujuan untuk mengetahui pengaruh penambahan berbagai media substrat mikroorganisme lokal (MOL) terhadap kandungan nutrien dan asam sianida fermentasi anaerobik kulit singkong. Penelitian dilaksanakan pada bulan September-Desember 2022 di Laboratorium Fakultas Pertanian Universitas Tidar, Laboratorium CV. Chemmix, Bantul, Yogyakarta, dan di Laboratorium Institut Pertanian Bogor. Penelitian menggunakan metode rancangan acak lengkap. Kulit singkong diberi 4 perlakuan dan 5 ulangan, yang terdiri dari (P0) kulit singkong + 1% Effective micro-organism EM4 1%; (P1) kulit singkong + 1% MOL1; (P2) kulit singkong + 1% MOL2; (P3) kulit singkong + MOL3. Data dari hasil penelitian dianalisis menggunakan analisis ragam, jika hasilnya berbeda nyata dilanjutkan menggunakan uji Dunnet. Hasil penelitian menunjukkan bahwa penambahan berbagai MOL memberikan pengaruh nyata ($P<0,05$) terhadap kandungan protein kasar dan HCN, namun tidak memberikan pengaruh ($P>0,05$) terhadap kadar air dan abu pada fermentasi kulit singkong. P3 menghasilkan fermentasi kulit singkong terbaik dengan kandungan protein kasar paling tinggi dan kadar HCN paling rendah.

Kata Kunci: Kulit singkong, air cucian beras, air rebusan kedelai, air limbah ampas tahu

ABSTRACT

Limited forage causes the need for alternative feed to meet the needs of livestock, one of the alternative feeds that can be used is fermented cassava peels. This study aimed to determine the effect of adding various media substrates of local microorganisms (MOL) on the nutrient and cyanide acid content of cassava peels anaerobic fermentation. The research was conducted in September-December 2022 at the Laboratory of the Faculty of Agriculture, Tidar University, CV. Chemmix Laboratory, Bantul, Yogyakarta, Bogor Agricultural Institute Laboratory. The study used a Completely Randomize Method. Cassava peels were given 4 treatments and 5 replications, namely (P0) cassava peels + 1% EM4; (P1) cassava peels + 1% MOL1; (P2) cassava peels + 1% MOL2; (P3) cassava peels + 1% MOL3. Data were analyzed using a Completely Randomized Design (CRD) and continued with the Dunnet test. The results showed that the addition of various MOL had a significant effect (P<0.05) on crude protein content and HCN content but had no significant effect (P>0.05) on water content and ash content in the anaerobic fermentation of cassava peels. P3 Produced the best cassava peel fermentation with the highest crude protein and the lowest HCN content.

Keywords: Cassava peels, rice-washing water, soybean-boiled water, tofu wastewater

INTRODUCTION

Feed is one of the important factors in the maintenance and productivity of ruminants. Increasing the productivity of ruminants must be followed by an increase in the supply of sufficient forage both in quality and quantity because forage is the main source of feed for ruminants (Sari et al., 2016). The limited amount of forage causes farmers to use other alternatives to fulfill animal feed needs. One way that can be done is through the utilization of household and industrial waste, including coconut water, rice-washing water, soybean-boiled water, and tofu wastewater as ingredients for making local microorganism media or MOL which is used as a starter in the fermentation process. Fermented feed ingredients can use agricultural waste, one of which is cassava peel.

Cassava peels are waste from cassava processing such as the fermentation industry, tapioca flour industry, and staple food industry. According to Richana (2013), cassava peels contain nutrients such as crude protein 1.50-3.70%, crude fat 0.80-2.10%, crude fiber 17.50-27.40%, moisture content 7.90-10.32%, ash content 44.00-59.00%, magnesium 0.12-0.24%, calcium 0.42-0.77%, and phosphor 0,02-0,10%. Cassava peels contain toxic antinutrients, namely cyanide, so the use of cassava peel as feed in livestock needs to be limited. Cyanide levels in cassava peels can be overcome by fermentation. Fermentation is one of the efforts made to increase the nutrient content of feed ingredients, especially the protein content, and can minimize the cyanide content in cassava peels.

Anaerobic fermentation of feed is the result of storage and fermentation of fresh feed under anaerobic conditions with the help of lactic acid bacteria. The anaerobic fermentation process requires a starter to optimize the degradation process of the content in cassava peels so that livestock can digest it easily. In addition, starters can also increase the nutritional value of anaerobic fermentation (Kurniawan et al., 2015). The price of starters in circulation is quite high compared to local microorganism starters, so farmers need an alternative starter with low prices and quality such as local microorganisms. According to Hadi (2019), local microorganisms or abbreviated as MOL are solutions containing micro and macro elements and the presence of potential bacteria as a bioprocess to break down organic matter and growth promoters.

The making of MOL media substrate can utilize the waste around, both industrial waste and household waste. Some materials as MOL media substrates in this study were rice washing water, soybean-boiled water, and tofu wastewater. The materials that were used contain bacteria or bacterial food sources so that they could be used as MOL. This study aimed to determine the effect of adding various media substrates of local microorganisms (MOL) on the nutrient and cyanide acid content of cassava peels anaerobic fermentation.

METHOD

The research was carried out from September to December 2022 at the Laboratory of the Faculty of Agriculture, Tidar University, Magelang City, Central Java. The analysis of the cyanide acid (HCN) content of the samples was carried out at CV. Chemmix Laboratory, Bantul, Yogyakarta, meanwhile the analysis of crude protein content was carried out at Dairy Nutrition Laboratory Bogor Agricultural Institute. The study materials were cassava peels, rice-washing water, soybean-boiled water, tofu wastewater, coconut water, rumen fluid, EM4, and molasses.

Rumen Fluid Collection (Rusman, 2017)

Rumen fluid was taken from the slaughterhouse in the Magelang District. Retrieval of fluid from cow rumen was done by filtration (filtering with cotton fabric) under low-temperature conditions. The filtered rumen solution was centrifuged at 10,000 g for 10 minutes at 4°C to separate the supernatant from the microbial cells and cell contents. Then, the supernatant was used as a source of crude enzyme.

Preparation of Local Microorganisms (MOL) Media Substrate (Siburian, 2019)

Jerry cans were prepared with a size of 5 L or using gallons, then filled with 500 g of rumen fluid. In the next step, molasses was added as much as 100 ml. Liquid according to each treatment was added as much as 1 liter into the jerry can. All ingredients that had been included were mixed evenly. When the previous step had been finished, the mixture was covered with plastic and tied using rubber and then fermented for 14 days. The complete treatment is as follows:

P0: Cassava peels + 1% EM 4 + 3% molasses

P1: Cassava peels + 1% MOL1 (500 g rumen fluid + molasses 100 ml + 1000 ml coconut water + 1000 ml rice-washing water) + 3% molasses

P2: Cassava peels + 1% MOL1 (500 g rumen fluid + molasses 100 ml + 1000 ml coconut water + 1000 ml soybean-boiled water) + 3% molasses

P3: Cassava peels + 1% MOL1 (500 g rumen fluid + molasses 100 ml + 1000 ml coconut water + 1000 ml tofu wastewater) + 3% molasses

Preparation of Cassava Peels Anaerobic Fermentation (Santi, 2012)

Fresh cassava peels were chopped to a size of 5 cm. After that, the cassava peels were weathered for 1-2 days until the moisture content was 60-70%. The chopped cassava peels weighed as much as 2 kg for each glass jar. Molasses was measured at 3% and replicated 4 times. EM4 solution was measured as much as 1%. Local microorganism media substrates such as rumen fluid, coconut water, rice-washing water, soybean-boiled water, and tofu wastewater were measured according to each treatment. After all, liquids were measured, 1% EM4 solution was mixed with molasses and chopped cassava peels for the first treatment.

Molasses and each local microorganism were mixed until homogeneous according to the next treatment. The mixture of molasses and local microorganisms was mixed with cassava peels on a plastic mat until homogeneous. Cassava peels that had been added with EM4, molasses, and local microorganisms were put into a glass jar and compacted. The compaction process was carried out by pressing to reduce air space and minimize the amount of oxygen, then the glass jar was tightly closed by adding solation around the glass jar lid. The material in the glass jar was allowed to ferment for 21 days or 3 weeks. On the 21st day, the glass jar was opened and evaluated for quality. The fermented cassava peels were then tested based on the content of crude protein, HCN, moisture, and ash.

Analysis of Nutrient and Antinutrient Content

Crude protein was analyzed by micro Kjeldahl AOAC (2005) method. 2 g of sample was weighed and then put into a 100 ml volumetric flask, added 10 ml of concentrated H_2SO_4 with 2 g of catalyst, and digested until the solution became clear, and continued to be destructed for 10 minutes. The clear solution was cooled, then diluted with 3 ml of distilled water, then added 5 ml of 45% NaOH. The next step is to add a few drops of PP indicator, then distillate. The distillation results were put into a 125 ml Erlenmeyer containing 10 ml of 2% boric acid (H_3BO_3) which contained 0.1% bromocresol green and 0.1% methyl red with a ratio of 2:1. Titration was carried out with 0.01 N HCl until the color of the solution in the Erlenmeyer turned pink. The titration volume was read and recorded. Crude Protein content was calculated by formulas:

$$\%CP = \frac{(HCL\ blank\ titration\ (ml) - HCL\ titration\ (ml)) \times N\ HCL \times 0{,}014 \times 6{,}25}{sample\ (ml)} \times 100\%$$

HCN was analyzed by Sudarmadji et al. (1997) method. The sample was weighed as much as 20 g, then added 10 ml distilled water into Erlenmeyer and left for 2 hours. Distilled water 10 ml was added and distilled over steam. Distillate is put into an Erlenmeyer flask which has been filled with 20 ml of 2.5% NaOH solution. The distillation process is prohibited when the distillation (accommodated in Erlenmeyer) reaches a volume of 150 ml, then added 5 ml Kl 5% and 8 ml NH_4OH during distillation. The distillate mixture is titrated with 0.02 N $AgNO_3$ solution until turbidity appears. HCN content was calculated by formulas:

$$Ppm\ HCN = \frac{AgNO3\ (ml) \times 0{,}54}{sample\ (g)} \times 1000\ mg/kg$$

The water content of cassava peel fermented was analyzed by AOAC (2005). The porcelain cup is dried in the oven at 100-105°C for 30 minutes or until a constant weight is reached, then cooled in a desiccator for 30 minutes, then weighed. The sample is weighed into a cup in an amount of 5 g, then dried in an oven at 100-105°C until a constant weight is reached. The sample was cooled in a desiccator for 30 minutes and then weighed. The percentage of water content can be calculated using the following formula:

$$(\%)\ Water\ content = \frac{sample\ (g) - sample\ after\ dried\ (g)}{sample\ (g)} \times 100$$

The ash content of cassava peel fermented was analyzed by AOAC (2005). The porcelain cup is dried in the oven at 100-105°C for 30 minutes or until a constant weight is reached, then cooled in a desiccator for 30 minutes, then weighed. The 5 g sample (100% dry matter) is weighed into a cup, then burned in the furnace at 400°C until it ashes. The furnace temperature is then increased to 600°C until complete ashing. The sample was then cooled in a desiccator for 30 minutes and then weighed. The calculation of ash content is as follows:

$$(\%)\ \text{ash} = \frac{porcelain\ with\ ash\ (g) - porcelain\ (g)}{sample\ (g)} \times 100$$

Research design and statistical analysis

The study used a Completely Randomize Method. Data were analyzed using a Completely Randomized Design (CRD) and continued with the Dunnet test. Data were calculated using IBM SPSS 23.

RESULT AND DISCUSSION

Crude Protein Content

The results showed that the provision of different MOL media substrates had a significant effect ($P<0.05$) on the crude protein content in the anaerobic fermentation of cassava peels. It indicated that the addition of local microorganisms (MOL) can change the crude protein content in the anaerobic fermentation of cassava peels. The difference in protein content is thought to be because local microorganisms contain proteolytic microbes that produce protease enzymes that break down proteins into polypeptides which then become simple peptides causing differences in protein compared to P0 as presented in Table 1. Proteolytic microbes can produce protease enzymes as protein breakers. Proteins will be broken down into polypeptides, then into simple peptides, then these peptides will be broken down into amino acids. Microbes will utilize the amino acids to multiply themselves. The number of microbial colonies that are the source of single-cell protein will increase during the fermentation process..

Protein consists of amino acids that function as body-building blocks. The protein requirement of ruminants is based on crude protein content. The measurement of crude protein in feed ingredients is based on an analysis that measures the amount of N in the feed ingredients. This is because microbes in the rumen can degrade proteins into peptide bonds and ammonia (NH_3) and organize them into essential and non-essential amino acids. Crude protein content after fermentation tends to increase due to microbes that have good growth and proliferation, which can change more constituent components derived from the microbial body itself to increase the crude protein content of the local microorganism media substrate.

The nutrient content in rice washing water, soybean-boiled water, and tofu wastewater is also thought to affect protein levels in cassava peel fermentation. Rice washing water contains more carbohydrates than other nutrients. According to the statement of Setiawati (2013), rice washing water contains carbohydrates in the form of starch 80-90%, crude protein, cellulose, hemicellulose, sugar, and high vitamins. Meanwhile, soybean-boiled water has a lower protein content than tofu wastewater. According to Suwardiyono et al. (2019), soybean-boiled water contains 0.11% carbohydrates, 0.42% protein, 0.13% fat, 4.55% iron, 1.74% phosphor, and 98.8% water. The results of Siburian's research (2019) showed an increase in the crude protein of 0.76-1.60% in cassava peels after fermentation using various MOL. The crude protein content ranged from 4.93-5.77%, smaller when compared to this study.

Dunnet's further test results showed that P1, P2, and P3 are significantly different compared to P0, as presented in Table 1. Based on the value, P3 has the most significant difference because it has the highest value and the highest difference from P0 (1.74). The media for local microorganism used in P3 is tofu wastewater. This is because the protein content in the tofu wastewater is 2.91% (Irawan, 2019). This value is bigger than soybean-boiled water at 0.42% (Suwardiyono et al., 2019), while rice-washing water is 1.7% (Tatsugawa et al., 2011). Crude protein in the tofu wastewater is thought to be able to affect the performance of bacteria, so they can multiply. In addition, the quality of N microbial is thought to be higher than the N in cassava peel. This causes the protein content to increase after the fermentation process. Besides that, differences in crude protein content in each medium are thought to affect the protein content in fermented cassava peels.

Moisture Content

The results showed that the provision of different MOL (Local Microorganisms) media substrates had no significant effect (P>0.05) on the moisture content of cassava peel anaerobic fermentation. The water content that was not different in this study was thought to be due to the provision of a small amount of MOL media substrate. Giving a high percentage of MOL media substrate will cause the water content to increase. This happens because the mass of microbes that help the cassava peel fermentation process will increase. Microbes need energy sources in the form of carbohydrates in fermentation. The by-product of this process is water. More microbial mass will cause the intensity of metabolism that takes place during fermentation to increase as well so that it will produce more water (Siburian, 2019). The increase in silage water content occurs due to the formation of CO_2, H_2O, and heat resulting from the digestion of glucose which is part of the dry matter in the early stages of fermentation during the aerobic phase (Septian et al. (2022b). The average water content in each treatment is presented in Table 1.

The water content in P1-P3 was not significantly different when compared to P0, meaning that cassava peel fermentation with MOL was able to maintain the same water content as P0. This is thought to be due to the influence of the amount of MOL media substrate given. Pratiwi et al. (2015) argued that due to the small dose of MOL media substrate used, the activity of microbes during the fermentation process did not affect the treatment. In contrast to the research of Septian et al. (2022a) which showed an increase in moisture content in coffee peel fermentation using Heryaki probiotics. This is due to the different percentages of probiotics given, while this study used MOL media substrate with the same percentage in each treatment. According to Septian et al. (2022a), along with the decrease in dry matter caused by microbial activity in utilizing organic matter as its energy needs, the moisture content in the fermentation process increases. In addition, the presence of water content in the fermentation is a result of the formation of lactic acid and a decrease in pH during the fermentation process. Lactic acid is liquid, so the liquid nature causes the moisture content in the fermentation process. The silage moisture content is affected by respiration and fermentation. Respiration will cause many nutrient contents to decompose so that it will reduce dry matter, while fermentation will produce lactic acid and water.

According to Purwaningsih (2015), good-quality silage contains a moisture content of 67%. This means that cassava peel fermentation using various MOL media substrates has a good moisture content, as presented in Table 1 that the moisture content ranges from 65-67%. Based on Siburian's research (2019), the moisture content of cassava peels fermented with various MOL media substrates is very low. However, there was an increase of 0,91-5,3%. The moisture content ranged from 6,91%-11,3%, much smaller than this study.

Ash Content

Ash content is used to determine the mineral content in a material because of the demineralization process. The lower the ash content, the higher the quality and purity of the material. Ash content measurement is one of the important parameters that need to be done to evaluate the nutrients and composition in a sample. Ash content testing in a furnace is done at 600ºC. Oxidation at high temperatures is done to find out how many mineral components are left behind. The process of analyzing ash content is carried out for several hours to get perfect results (Sunartaty and Yulia, 2017).

The results showed that the provision of different MOL media substrates did not give a significant effect (P>0.05) on the ash content of cassava peel anaerobic fermentation. These results are thought to be due to the utilization of mineral elements contained in the ash. It is suspected that lactic acid bacteria contained in the silage process require minerals only in small amounts to support their growth and development. This small need for minerals can cause an unreal effect on ash content. Reed (1975) also stated that fermentation treatment had no effect or showed no significant effect on the ash content (inorganic material) of the substrate. The average ash content of each treatment is presented in Table 1.

Ash content can be influenced by the amount of organic matter contained in the material. Siburian (2019) added that the less organic matter that is degraded, the less the increase in ash content, conversely the more organic matter that is degraded, the more the increase in ash content. The results of cassava peel fermentation showed ash content that was not significantly different from P0, stating that fermentation with MOL media substrate had the same quality as fermentation using EM4 in maintaining ash content. This indicates that the organic matter in the material is not overly broken down. As explained earlier, lactic acid bacteria only need small amounts of minerals in the fermentation process (Noviana et al., 2012). In addition, the presence of minerals in a substrate cannot be eliminated. Each local microorganism media substrate will still have mineral content even though it has been burned. The provision of local microorganism media substrate in liquid form with the same percentage between treatments caused the ash content of cassava peels after fermentation to not differ significantly.

According to Ringgita et al. (2015), the ash content of ruminant feed should not be more than 15%. The results of this study showed that the ash content ranged from 5.59-5.63%. This means that cassava peel fermentation with MOL media substrate is suitable for livestock use. Meanwhile, Siburian (2019) research showed ash content results that were not much different. The ash content of cassava peel fermentation with various MOL decreased by 0.69-2.41%, with values ranging from 4.31-6.09%.

Cyanide Acid Content

Cyanide is a chemical compound from the Cyano group, which consists of 3 carbon atoms bonded with nitrogen (C = N) and combined with other elements such as potassium or hydrogen (Cahyawati et al., 2017). According to Erlina (2006), the type of microbes that play a role in the degradation of cyanide compounds is the type of *Pseudomonas* that will degrade cyanide into formate and ammonia or by releasing ammonia and other compounds. Cyanide in small concentrations can be degraded by certain microbes into nitrogen gas (Pitoi, 2015).

The results showed that the provision of different MOL media substrates had a significant effect (P<0.05) on HCN levels in the anaerobic fermentation of cassava peels. The average HCN content of each treatment is presented in Table 1. The research showed HCN levels in cassava peels decreased after fermentation with MOL. The decrease in cyanide acid levels in cassava peels with fermentation treatment is closely related to the production of linamarase enzyme produced by microbes in the MOL media substrate to destroy linamarin and lotaustralin which are cyanogenic glucoside compounds that are dangerous when consumed in large quantities. This enzyme degrades linamarin into acetone cyanohydrin, which spontaneously decomposes into water-soluble and volatile cyanide acid (HCN) (Siburian, 2019).

Sandi et al. (2013) stated that cassava peels contain 109 ppm of cyanide acid. Cyanide acid content greater than 100 ppm indicates that cassava peels are harmful to livestock that consume them. Based on this study, the HCN content of cassava peels after fermentation using MOL media substrate is less than 100 ppm, so giving fermented cassava peels with MOL media substrate to livestock can be done. This is to research conducted by Simbolon et al. (2016), it is known that there is a decrease in cyanide acid levels in cassava peels with fermentation treatment. Before fermentation, cyanide acid levels were known to be 580.93 ppm, down to 1.16 ppm after fermentation. Cyanide acid is lost when chopped and dissolves in the addition of water in the treatment. Enzymes in the fermentation process are also a factor in the loss of cyanide acid in cassava peels. Hidayat (2009) added that fermentation is one of the treatments that can reduce cyanide acid levels. This is due to the influence of microbes that can degrade cyanide acid in small concentrations into nitrogen gas.

The most significantly different treatments were shown by P2 and P3 because they had the smallest number, and each has a different value of -8.37 and -12.98 with P0. The substrate used for the growth of local microorganisms in P2 is soybean-boiled water, while P3 uses tofu wastewater. This is thought to be due to the presence of several microbes in soybean-boiled water and tofu wastewater. Based on research by Nurwahidah and Alif (2022), soybean-boiled water can be a media substrate for the development of *Pseudomonas fluorescens* bacteria. According to Simbolon (2018), tofu wastewater contains protein, fat, vitamins, and minerals (Ca, Mg, Fe) so it has the potential as a medium for microorganism growth. Some microorganisms that grow in tofu wastewater include *Streptococcus* sp., *Enterobacter* sp., and *Pseudomonas* sp. MOL media substrate with soybean-boiled water and tofu wastewater contains *Pseudomonas* bacteria that can degrade cyanide compounds found in cassava peels.

Table 1. The nutrient content of anaerobic fermentation of Cassava peel

Variables	Treatments			
	P0	P1	P2	P3
Crude Protein (%)	9.60±0.16	10.47±0.22 (0.87)*	10.70±0.15 (1.11)*	11.33±0.21 (1.74)*
Water Content (%)	65.15±1.22	67.95±0.63	65.59±1.59	67.83±0.82
Ash Content (%)	5.04±0.36	5.59±0.23	5.87±0.28	5,63±0.27
Cyanide Acid (ppm)	38.39±1.21	34.02±1.41 (-4.37)*	30.02±0.51 (-8.37)*	25.41±0.41 (-12.98)

Note: * = significantly different; the number inside (…) denotes the distance difference with control (P0)

CONCLUSION

Based on the results of the research, it can be concluded that anaerobic fermentation of cassava peel using MOL media substrate has a significant effect (P<0.05) on increasing crude protein content and decreasing cyanide acid content, but it has no significant effect (P>0.05) on water content and ash content. MOL made from rice-washing water, soybean-boiled water, and tofu wastewater can replace commercial probiotics like effective microorganisms (EM4). P3 Produced the best cassava peel fermentation with the highest crude protein (11.33%) within 1.74% of P0 and the lowest HCN content (25.41%) within -12.98% of P0.

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