EVALUATION OF STORAGE LENGTH ON THE NUTRIENT QUALITY OF WIN PROB FERMENTED WOODS (OTW_PRO) IN LIQUID FORM

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Abstract: *The aim of this research is to find out how long the best storage time for the fermentation process is on the quality of Win Prob (otw_pro) fermented onggok nutrients in liquid form. The hypothesis of this research is to find out how long the best storage time for the fermentation process is on the quality of Win Prob (otw_pro) fermented onggok nutrients in liquid form. The materials used in the research were onggok, Win Prob and molasses. Materials for chemical feed nutrition tests are H2SO4, NaOH, acetone, boric acid HCl and methyl red indicator. The tools used are plastic jars, buckets, knives and scales. The tools used in nutritional chemistry tests are calorie meter bombs, thermometers, digestion tools, distillation tools, titration tools, and shoxlet tools. The research method used in the research was a non-factorial Completely Randomized Design (CRD) with 5 treatments and 4 replications. The treatments given were as follows: FP0: storage time 0 days (control), FP1: storage time 1 week, FP2: storage time 2 weeks, FP3: storage time 3 weeks and FP4: storage time 4 weeks. The parameters observed include analysis of the nutritional content of crude protein, crude fiber and gross energy. Proximate testing is carried out in the laboratory. The conclusion of this research is that onggok fermented with Win Prob probiotics in liquid form with a storage period of 4 weeks is the best treatment which can increase crude protein, gross energy and reduce crude fiber*

INTRODUCTION

Livestock has an important role in meeting people's animal protein needs (Siregar, 2018). Feed is the most important aspect and is the largest variable cost in livestock operations. Demand for animal feed continues to increase, but is often not in line with the availability of local feed raw materials. Therefore, imports are the main choice to meet domestic animal feed needs. Even though Indonesia has abundant agricultural industrial

waste resources, such as waste from tapioca production which comes from cassava, to date its potential has not been utilized optimally.

Currently, Indonesia is the fourth largest cassava producing country in the world. Respectively, there are Nigeria with 57 million tons, Thailand with 30 million tons, Brazil with 23 million tons and Indonesia with 18-20 million tons. Indonesia has great potential in utilizing cassava as raw material for the animal feed industry. Cassava production in Indonesia reaches 18.3 million tons per year (BPS, 2022). Cassava is generally used in the traditional food industry, such as getuk, thymus, chips, gemblong, and various other types of food. One of the by-products of processing cassava into tapioca flour is onggok, which is produced in significant quantities, ranging from 10% to 15% of the weight of fresh cassava processed.

However, large ongok production can become an environmental problem if not managed properly. Therefore, processing onggok into alternative feed is a good solution. However, onggok has problems with its nutritional value, especially its low protein content. Therefore, a processing technique is needed that can improve the nutritional quality of the onggok. One proposed solution is through fermentation technology.

Fermentation technology is the process of storing a substrate in an anaerobic condition by adding minerals, embedding microbes in it, followed by incubation at a certain temperature and time with the aim of increasing the nutritional value, especially protein levels and reducing fiber levels. The use of fermentation technology to increase the nutritional value of agricultural waste as an alternative feed source can help solve the problem of shortages of animal feed ingredients and the problem of unutilized waste (Pasaribu, 2007).

Efforts to process feed through a fermentation process are able to break down complex organic compounds into simpler compounds so that they can be digested well by livestock. This fermentation process resembles feed fermentation activity in the intestines of livestock, so that if this waste is fermented to be used as feed, it is hoped that it can increase digestibility efficiency and increase feed nutrient retention. Fermented substrates usually have a higher nutritional value than the original material, due to the catabolic and anabolic properties of microorganisms so they are able to break down more complex components to make them easily digestible. This process is expected to renovate the chemical network structure of cell walls, break lignocellulose bonds and reduce lignin levels (Moningke et al., 2022).

Onggok fermentation technology aims to increase the nutrient content of onggok so that it can become a high quality alternative feed. Onggok, as a fermentation medium, is rich in carbohydrates but requires additional ingredients to support mold growth. The use of onggok mixed with urea, zeolite, and fermented with the mold Aspergillus niger has been proven to increase the crude protein and pure protein content and reduce the high crude fiber content in onggok. The addition of ammonium sulfate, as proposed by Pitriyatin (2010), can also increase protein content and improve amino acid composition, making it a source of sulfur for the amino acids methionine and cystine.

Onggok fermentation technology with probiotics, such as Win Prob, is an innovation that aims to maximize the use of onggok as animal feed through a fermentation process catalyzed by beneficial microorganisms. Win Prob probiotics, as a fermentation agent

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enriched with beneficial microorganisms, are key in improving the quality of onggok. These probiotic microorganisms, such as lactic acid bacteria or other types, play a role in changing the nutritional composition of onggok. The fermentation process can increase protein content, reduce crude fiber, and increase the availability of other nutrients.

Win Prob is a fermentator that contains cellulolytic microorganisms, including the fungus Aspergillus niger and Trichoderma viride. The microorganisms contained in the win prob include Aspergillus niger, Bacillus subtilis, Lactobacillus aeidophyllus, Rhizopus oligosporus, Saccharomyces cerevisiae and Trichoderma viridae (Rahayu and Subagyo, 2019). Nurhadianty et al. (2018) stated that the fungi Aspergillus niger and Trichoderma viride are microorganisms from a type of fungus that are considered safe and by the Food and Drug Administration (FDA) in America, these fungi are classified as Generally Recognized as Safe (GRAS) microbes.

Based on the description above, the researcher wants to conduct research on the evaluation of storage time on the quality of Win Prob Fermented Onggok Nutrients (Otw_Pro) in liquid form.

RESEARCH METHODS

This research was carried out at the Panca Budi Development University Laboratory, Building C. The research period is from December 2023.

The materials used in the research were onggok, Win Prob and molasses. Materials for chemical feed nutrition tests are H_2SO_4, NaOH, acetone, boric acid HCl and methyl red indicator. The tools used are plastic jars, buckets, knives, and scales. The tools used in nutritional chemistry tests are calorie meter bombs, thermometers, digestion tools, distillation tools, titration tools, and shoxlet tools.

The research method used in the research was a non-factorial Completely Randomized Design (CRD) with 5 treatments and 4 replications. The treatment given is as follows:

FP0: storage time 0 days (control)
FP1: storage time 1 week
FP2: storage time 2 weeks
FP3: storage time 3 weeks
FP4: storage time 4 weeks

RESEARCH IMPLEMENTATION

Research Implementation

Manufacturing Procedure

The first stage is to prepare all the ingredients, then weigh them based on the percentage of use. Pour all the ingredients above the container starting from the material with the highest percentage of use in the bottom layer, namely onggok, while for probiotics (Win Prob) dissolve it in water that has been mixed with molasses (the amount of water used is ± 30 percent of the total weight of onggok) then Water that has been mixed with molasses and Win Prob is poured over the pile of ingredients, then stirred using your hands until it is homogeneous (even). After that, put it in a plastic jar, compact it by pressing, then close the jar tightly and ferment for a long time of storage according to

anaerobic treatment.

Analysis Sampling

Samples for chemical analysis of nutritional content were taken randomly based on treatment. Sampling started from the beginning of the onggok production before fermentation (P0), 1 week after fermentation (FP1), 2 weeks after fermentation (FP2), 3 weeks after fermentation (FP3) and 4 weeks after fermentation (FP4). The samples that have been taken are immediately dried (drying in the sun/in an oven at 60 degrees Celsius), then the samples are weighed and ground with a blender for later analysis in the laboratory.

Research Parameters

The parameters observed in this research were, analysis of the nutritional content of crude protein, crude fiber and gross energy in fermented onggok using Win Prob (OTW_PRO) in liquid form. Proximate testing is carried out in the laboratory.

1) *Analysis of Crude Protein Content*

⇒ *Destruction Stage*

At this stage the sample is weighed first, after that it is put into a Kjeldahl flask then concentrated sulfuric acid (H2SO4) and a catalyst are added, then digested at a temperature of 410°C continuously until the solution is clear in color, then let the solution sit and wait until it cools. The results at this stage will then proceed to the distillation stage.

⇒ *Distillation Stage*

After the digestion stage, the solution is then put into a distillation flask and then added with distilled water and NaOH. The solution was then collected using an Erlenmayer containing a standard acid solution.

⇒ *Titration Stage*

The solution from the distillation stage is titrated using HCl solution until the color of the solution changes color.

Crude protein content is calculated using the formula :

$$\text{Crude protein content (\%)} = \frac{(VA - VB) \times N\ HCl\ s\ 14{,}007 \times 6{,}25}{\text{sample weight (g)} \times 1000} \times 100\%$$

Note: VA = milliliter titration for sample
VB = military titration for blank
N = HCl concentration used
14.007 = atomic weight of nitrogen
6.26 = Conversion factor

2) *Analysis of Crude Fiber Content*

⇒ *Determination of crude fiber content refers to AOAC 2005 by weighing a sample of 1 gram and placing it in a 300 mL Erlenmeyer flask, then adding 100 mL of 0.3N H2SO4 and boiling under refrigeration, turning*

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over for 30 minutes.

⇒ *After boiling, add 50 mL of 1.5 N NaOH and filter again for 30 minutes.*

⇒ *The liquid in the Erlenmeyer flask is filtered using filter paper of known weight.*

⇒ *Filtering is carried out using a vacuum pump and then washed using a vacuum pump. Wash successively with 50 mL hot water and 25 mL acetone.*

⇒ *The residue and filter paper are dried until the weight is constant and then calculated by weighing:*

$$\text{Crude fiber content} = \frac{A - B}{W} \times 100\%$$

Note: A = residue weight in dried filter paper (g)
B = weight of empty filter paper (g)
W = sample weight (g)

3) Gross Energy Analysis

⇒ *The calorific value or gross energy of feedstuffs is measured using a bomb calorimeter by joining the electrode tip to the burner wire.*

⇒ *The weighed sample is then put into the combustion bowl and then placed on the electrode support. Attach the bomb cap with the container until it is tightly attached and tight.*

⇒ *The bomb vessel was filled with oxygen gas for 1 minute by turning on the Fill menu on the device monitor.*

⇒ *The bomb vessel is inserted into a water vessel that has been filled with distilled water as much as 2 liters first. The water vessel was then put into the jacket container and tightly closed using the bomb bucket.*

⇒ *The electrode cable is then connected to a 23 V power supply and press the Start button. Wait until the stirring process is complete or approximately 5 minutes. At the 6th minute, the temperature was recorded with the code t1.*

⇒ *The power supply button is turned on so that combustion occurs in the bomb. Observe the temperature change until the temperature stabilizes again and then record the temperature again and coded as t2.*

⇒ *Crude protein content is calculated using the formula:*

Description: VA = milliliter titration for sample
VB = military titration for blank
N = HCl concentration used
14.007 = Atomic weight of nitrogen
6.26 = Conversion factor

RESULTS AND DISCUSSION

The recapitulation of the nutritional content (crude protein, crude fiber, gross energy) in onggok fermented with Win Prob (otw_pro) in liquid form is presented in Table 1.

Table 1. Recapitulation of average nutritional content (crude protein, crude fiber, gross energy) in onggok fermented with Win Prob (otw_pro) in liquid form.

Treatment	*Parameters*		
	Crude Protein (%)	Crude Fiber (%)	Gross Energy (cal/100g)
FP0	2,12[a]	31,81[tn]	3119,47[tn]
FP1	2,27[a]	30,45[tn]	3237,84[tn]
FP2	2,36[a]	29,52[tn]	3310,31[tn]
FP3	2,69[b]	26,52[tn]	3399,61[tn]
FP4	2,93[c]	25,59[tn]	3465,57[tn]

Notes: Different superscripts in the same column indicate significantly different results (p<0.05).
tn = not significantly different

Crude Protein

The results of analysis of variance showed that treatment of onggok fermented by Win Prob (otw_pro) in liquid form with different incubation times of 0 days to 4 weeks had a significant effect (P<0.05) on crude protein content.

The results of research on onggok fermented by Win Prob (otw_pro) in liquid form with different incubation times of 0 days to 4 weeks in different treatments with fermentation times of up to 4 weeks on crude protein content showed that the average crude protein content was 2.12 % (FP0); 2.27% (FP1); 2.36 % (FP2); 2.69% (FP3); and 2.93% (FP4). The highest average crude protein content was obtained in treatment FP4 (Win Prob (otw_pro) fermented onions in liquid form with an incubation period of 4 weeks) followed by FP3, FP2, FP1 and FP0.

Win Prob is a probiotic that can be used in making fermented animal feed. According to Zuliagus' statement. (2017) probiotics containing Aspergillus niger, Bacillus Subtillus, Rhizopus Oligos porus Trichoderma Viride, Saccharomyces cerevisiae which produce cellulase enzymes Probiotics are microorganisms that can increase the growth and efficiency of animal feed without causing the absorption of probiotic components in the animal's body so that there are no residues and no mutations occur in livestock.

Win Prob Livestock is a probiotic specifically designed for ruminant animals such as cows, goats and sheep. It is specifically recommended for closed fermentation of feed with the aim of reducing crude fiber and very coarse fiber. Win Prob is a fermentator that contains cellulolytic microorganisms, including the Aspergillus niger fungus (Sri Rahayu, 2021).

Cellulolytic microorganisms, including the fungus Aspergillus niger, have the ability to degrade cellulose. Cellulose is a complex polymer that is the main component of plant cell walls. This cellulose degradation process is important in the production of cellulase enzymes which can break down cellulose bonds into simple sugars. (Palinggi et al., 2008)

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stated that the Aspergillus niger fungus is a microorganism from a type of fungus that is considered safe and by the Food and Drug Administration (FDA) in America, this fungus is classified as a Generally Recognized Safe (GRAS) microbe. A technology that can degrade lignin bonds with cellulose and hemicellulose is by breaking down the components through a degradation or fermentation process using microbial activity (Kusnadi, 2008). In this way, the fermentation treatment is expected to be able to improve the quality of sugarcane bagasse waste for the better.

According to research from (Sri Rahayu, 2021) that giving Win Prob for sugarcane bagasse fermentation has a real influence on the physical quality, color, texture and pH characteristics, but has no real influence on the aroma characteristics. The best treatment was found in A3 (giving 15% Win Prob).

Crude Fiber

The data in table 1 shows that the average crude fiber content in onggok that underwent fermentation using Win Prob over a period of 0 days to 4 weeks was as follows: 31.81% (FP0); 30.45% (FP1); 29.52% (FP2); 26.52% (FP3); and 25.59% (FP4). Treatment FP0 produced the highest level of crude fiber, followed by FP1, FP2,F P3, and FP4. However, there was no statistically significant difference between the five treatments based on the results of analysis of variance (P>0.01).

The P0 treatment has a higher crude fiber content value because there is no onggok fermentation process with winprob (0 days) it is just mixed. Meanwhile, in treatment FP4, the crude fiber content value was lower compared to treatments FP1, FP2, and FP3. This is caused by the activity of Aspergillus niger contained in Win Prob in the FP4 treatment which can produce cellulase enzymes which can reduce the fiber content, and can degrade the fiber components in the onggok mixture into simpler compounds.

Win Prob, as a probiotic, also contains protease, lipase and pectinase enzymes (Melati et al., 2012). In contrast, pectinase can be produced from a variety of sources including bacteria, yeasts, and fungi. Various microorganisms such as Saccharomyces, Aspergillus, and Rhizopus have been proven capable of producing this pectinase enzyme (Mujdalipah, 2016.). Therefore, both types of probiotics contain microbes that are capable of producing protease enzymes to break down proteins and pectinase enzymes to digest carbohydrates.

According to research conducted by Indrayanti and Rakhmawati (2013), Aspergillus niger fermentation is recognized to produce a number of important enzymes such as amylolytic, cellulase, proteolytic and lipolytic, which in turn improve the nutritional quality of waste. These enzymes are responsible for breaking down the fiber components in the substrate into simpler compounds that can be utilized by the fungus to enrich the body's own metabolic processes. Because of its ability to produce various enzymes, Aspergillus niger is often used widely in commercial industry, especially in the production of citric acid, gluconic acid, and the manufacture of various types of enzymes such as amylase, pectinase, amyloglucosidase, and cellulase. Aspergillus niger has the ability to grow quickly and can grow in a wide temperature range, namely optimal at 35-37ºC, minimum at 6-8ºC, and maximum at 45-47ºC, with the need for adequate oxygen

(aerobic).

Gross Energy

Gross Energy is the total energy contained in a fuel or organic material before it undergoes a metabolic process. This includes all the energy contained in the chemical bonds within the material, including the energy released when the material burns completely to form carbon dioxide (CO_2) and air (H_2O). Gross energy calculations are important in determining the nutritional value of food and feed, because they provide an overview of the energy potential of the material.

This energy is not directly absorbed from food, but comes from the oxidation process of food substances such as carbohydrates, fats and proteins during the body's metabolic processes. Also known as gross energy, this energy is the total energy contribution from the feed consumed by livestock. Some of this gross energy is wasted in the form of feces and urine, while the remainder is used as metabolic energy to support body functions (Sumadi, 2017).

Based on the results of the analysis of variance, it shows that the onggok fermented by Win Prob with a long fermentation time of 0 days to 4 weeks gave results that were not significantly different (P>0.01) to the gross energy content of onggok. The average gross energy content of FP0, FP1, FP2, FP3 and FP4 respectively is 3119.47cal/100g; 3237.84cal/100g; 3310.31cal/100g; 3399.61cal/100g; and 3465.57cal/100g.

CONCLUSION

Onggok fermented with Win Prob probiotics in liquid form and a fermentation time of 4 weeks can increase crude protein and gross energy content and reduce crude fiber in onggok.

SUGGESTION

To achieve more optimal results, future researchers are advised to continue research on the best treatments identified in this study. The focus can be directed towards the application of these treatments to livestock to understand their growth response and digestive efficiency with the aim of providing the best recommendations to the public.

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