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Research Article

Fungal Fermentation of Cassava Stems with *Pleurotus ostreatus* and *Trichoderma asperellum* for Enhanced Ruminant Feed Quality

Agung Kusuma Wijaya[1], Kusmartono[2*], Hartutik[2], Mashudi[2]

[1]Department of Animal Science, Faculty of Agriculture, Lampung University, Lampung, Indonesia; [2]Department of Animal Nutrition, Faculty of Animal Science, Brawijaya University, Malang, Indonesia.

Abstract | This research aims to evaluate the effect of using *Pleurotus ostreatus* and *Trichoderma asperellum* fermented cassava stems on physical properties and chemical quality. The research was arranged in a factorial Completely Randomized Design (CRD), with 2 types of fungi (*Pleurotus ostreatus* and *Trichoderma asperellum*) and three levels applied (0, 3, and 6% for *Pleurotus ostreatus* and 0, 1.5, and 3% for *Trichoderma asperellum*). Therefore, 3 x 3 treatments with 3 replications were used. Based on the analysis of variance in the evaluation of physical quality, it showed that the combination did not show interaction (p>0.05) on physical properties. The use of 6% *Pleurotus ostreatus* produces a brown color and a normal pH value of 7.23. Meanwhile, interactions did not give any significant effects (p>0.05) on nutritional content. The use of 6% *Pleurotus ostreatus* with 3% *Trichoderma asperellum* was able to increase crude protein to 7.86%, while 6% *Pleurotus ostreatus* with 1.5% *Trichoderma asperellum* was able to reduce crude fiber to 33.57% and increase TDN to 58.85%. There was a slight increase in crude protein and a reduction in crude fibre due to the treatments. This study found that fermenting cassava stems using fungi can be a potential source of feed for ruminants, but further studies on digestibility value need to be done in the future.

Keywords | Fermented cassava stems, Physical quality, Chemical quality, *Pleurotus ostreatus*, *Trichoderma asperellum*, Combination

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***Correspondence** | Kusmartono, Department of Animal Nutrition, Faculty of Animal Science, Brawijaya University, Malang, Indonesia; **Email:** kusmartono_ansi@ub.ac.id
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INTRODUCTION

Utilization of organic agricultural and plantation waste is one of the efforts to reduce the potential environmental impact of waste. Cassava plants (*Manihot esculenta*) are a food-crop commodity that has quite potential in Indonesia alongside rice and corn. In terms of cassava harvest, Indonesia is ranked 6th in the world with cassava production of 17.213.042 tons in 2023 (FAO, 2023). Cassava offers a variety of very promising and sustainable derivative products, both food and non-food. Therefore, as a non-food ingredient, cassava production produces several agricultural wastes with potential for use as ruminant feed after being processed (Silva *et al*., 2022).

Cassava stems are by-products that are not widely used and typically become waste, negatively affecting land quality (Serpa-Fajardo *et al*., 2022). Cassava stems have a biomass production of 50% of the tubers after separating tubers and leaves (Vong *et al*., 2025). Only a small amount of cassava stems are used as replanting material, and the rest become waste on the land (Hang and Preston, 2019). Stems are cassava plant waste products with the potential to be used as ruminant livestock feed, despite their low quality due to low crude protein content and high crude fiber. The protein content of cassava stems can reach 5.24%, carbohydrate 42.99%, dry matter 98.77%, ash 6.43%, lipid 1.37% and crude fiber 39.51% (Jumare *et al*., 2024). The fiber components in cassava stems contain a significant amount of cellulose, hemicellulose, and lignin. The use of waste as animal feed can reduce production costs and also maintain sustainability (Soares *et al*., 2024).

Efforts to increase the utility value of feed and eliminate the limitations of feed ingredients can be done by fermentation treatment. Harvesting forage, agro-industry waste or food product waste are easily rotten, and complete feed is frequently fermented to ensure stability, level of preference and feed safety are maintained during storage (Wang *et al*., 2023). The feed given to ruminants should be in good nutritional condition and at a safe level. The technique of fermenting cassava stems using fungi is aimed at increasing digestibility, as fungi may produce enzymes such as cellulase, hemicellulase that can degrade fibers. The choices of fungi to degrade crude fiber components are based on several provisions, including non-toxicity, easy application, low cost, and good product quality.

Pleurotus ostreatus is a fungus capable of degrading lignin found in feed ingredients. The enzymes produced by this fungus consist of peroxidase and lactase; laccase collaborates with the peroxidase to further degrade lignin and other phenolic compounds (Ramamurthy *et al*., 2024). Other fungi that can be utilized are mushrooms. *Trichoderma asperellum*, a type of fungus often found in all soil types and various habitats. This fungus produces cellulase enzymes and is used as a starter in fermentation using the solid-state fermentation method on agricultural waste such as wheat straw, rice straw, wheat bran, corn cob, corn stover, cotton stalks, and sugarcane bagasse (Lima *et al*., 2024). Isolate of *Trichoderma sp.* used to decompose the fiber by producing higher decomposing enzymes that have cellulase, endoglucanase, xylanase, and laccase enzyme activities (Sarangi *et al*., 2021).

The use of *Pleurotus ostreatus* and *Trichoderma asperellum* is expected to break down lignocellulose and can degrade cellulose. Therefore, this research on cassava stems is very important because it has a strong fiber fraction. Research on the combination of *Pleurotus ostreatus* and *Trichoderma viridae* fungi has been carried out by Hatta *et al.* (2014) on the use of palm kernel meal for poultry. Currently, there has not been much research into the fermentation of cassava stems using a combination of two fiber-degrading fungi as ruminant feed. The research aims to evaluate is to evaluate the effect of using *Pleurotus ostreatus* and *Trichoderma asperellum* as a source of enzyme on the physical properties and nutritional value of cassava stems. This research only conducted physical quality and nutritional quality experiments to determine the potential of cassava stems before being given to animals.

MATERIALS AND METHODS

Experimental Site and Materials

This research was conducted from September to November 2023, with the incubation stage carried out at the Animal Nutrition and Feed Laboratory, Animal Husbandry Department, Faculty of Agriculture, University of Lampung. The proximate analysis was carried out at the Animal Nutrition and Feed Laboratory, Faculty of Animal Husbandry, University of Brawijaya. The materials used in this study were cassava stems (*Manihot esculenta*) obtained from South Lampung Regency, Lampung Province. *Pleurotus ostreatus* was obtained from farmers in South Lampung Regency and *Trichoderma asperellum* obtained from the Biotechnology Laboratory, Faculty of Agriculture, University of Lampung (Figure 1).

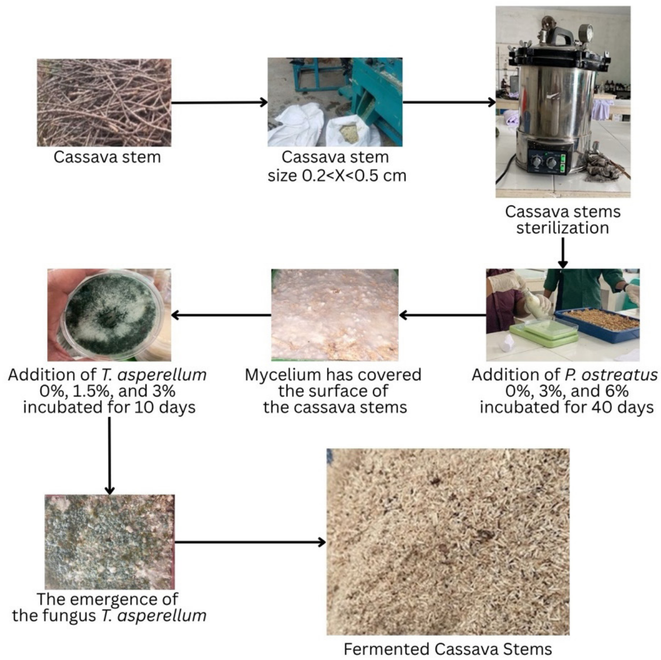

Figure 1: The creation of fermented cassava stems.

Experimental Method

The research method used was an experimental study using a factorial Completely Randomized Design (CRD), with 3 x 3 combinations of fungi repeated three times. The research material consisted of cassava stems fermented using a combination of fungi as factors. The first factor was *Pleurotus ostreatus* with levels of 0%, 3% and 6% incubated for

40 days. This dose is by research conducted by Manu *et al.* (2022), that with a 2% to 5% use of *Pleurotus ostreatus,* can increase the CP content of old coconut fiber. This is following research by Saputra *et al.* (2024), *Pleurotus ostreatus* was incubated in cassava stem media for 40 days. *Pleurotus ostreatus* needs time to break down nutrients in the media, so the time needed to grow optimal mycelium is 30-40 days after inoculation (Ridwan *et al*., 2023). The second factor is *Trichoderma asperellum* and with levels of 0%, 1.5% and 3% incubated for 10 days. The dose of *Trichoderma asperellum* according to research by Sunardi *et al.* (2023) that the use of *Trichoderma asperellum* at levels of 0% to 5% can increase CP in the fermentation of peanut straw. This is following Jumadi *et al.* (2021) that *Trichoderma sp*. incubation is carried out for 7 to 10 days before being used. The addition of the combination of fungi was carried out at different times. *Pleurotus ostreatus* was added first because this fungus requires a longer time for hyphae growth, then *Trichoderma asperellum* was added and incubated for a shorter time. The cassava stems have a moisture of 55,17% and were incubated at a temperature of 28 °C. The combination treatments are as follows (Table 1):

Table 1: Combination of fermentation using *Pleurotus ostreatus* and *Trichoderma asperellum.*

***Pleurotus ostreatus* (%)**	***Trichoderma asperellum* (%)**		
	0	**1.5**	**3**
0	Po_0Ta_0	$Po_0Ta_{1,5}$	Po_0Ta_3
3	Po_3Ta_0	$Po_3Ta_{1,5}$	Po_3Ta_3
6	Po_6Ta_0	$Po_6Ta_{1,5}$	Po_6Ta_3

Po: *Pleurotus ostreatus*; **Ta:** *Trichoderma asperellum.*

Data Collection

Physical quality: Assessment of the physical quality of fermented cassava stems was carried out using a sensory assessment method with trained panelists (n=10) and was carried out by blind test. This process was approved through an ethical approval letter, number 115/EC/KEPK-S3/05/2025. Observations were made to determine the feed quality based on texture, color, aroma, presence of mucus, and presence of fungi. The feed material assessment scale method was adapted from Sulistiyanto *et al.* (2024) as follows:

- Odor index on a scale ranging from 1 to 5, as 1 = rotten, 2 = odorless, 3 = a bit rancid, 4 = a bit rancid, starting to smell of fermentation, and 5 = smell of fermentation
- Color index on a scale ranging from 1 to 5, as 1 = dark brown, 2 = light brown, 3 = cream, 4 = light green, and 5 = dark green
- Texture index on a scale ranging from 1 to 5, as 1 = lumpy, 2 = slightly lumpy and smooth, 3 = Lumpy and moist, 4 = crumb, and 5 = no lumps and smooth
- Moldy index on a scale ranging from 1 to 5, as 1 = at all observation points, 2 = at most observation points, 3 = Half of the observation points, 4 = A little observation point, and 5 = not found
- Mucus index on a scale ranging 1 to 5 as 1 = at all observation points, 2 = At most observation points, 3 = Half of the observation point, 4 = A little observation point and 5 = not found

pH: acidity (pH) was measured using a pH meter that has been calibrated with buffer solutions at pH 4 and 7. Fermented cassava stems were ground and dissolved in a distillate water with a ratio of 8:10. The pH meter was then inserted to determine the pH.

Temperature: Temperature measurement during the fermentation process was conducted using a thermometer. Cassava stems were measured during 20-30 days of incubation by inserting a thermometer into the fermentation medium. This is because the fermentation process is characterized by an increase in temperature at the optimal time.

Chemical quality: Chemical quality parameters were determined by calculating nutrient content such as dry matter, organic matter, crude protein, crude fat, crude fiber, Non-nitrogen Free Extract (NFE), and total digestible nutrient (TDN), based on the instructions of AOAC (2005).

Data Analysis

The data from this research were tabulated using Microsoft Excel. The physical and chemical quality of fermented cassava stems was analyzed using analysis of variance (ANOVA) in Genstat software. Significant test results from treatment differences were followed by the Duncan Multiple Range Test (DMRT) to determine the differences between treatments. The observations obtained were analyzed as described by Gaspersz (1992). The statistical model for the analysis was as follows:

$$CRD\ \gamma_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

When;

i: 0, 3, and 6 (*Pleurotus ostreatus* inoculum levels).

j: 0, 1.5, and 3 (*Trichoderma asperellum* inoculum levels).

μ: The overall mean response.

α_i: The effect due to the i-th level of factor.

β_j: The effect of inoculum level on k-j.

$(\alpha\beta)_{ij}$: The effect of interaction between inoculum doses of *Pleurotus ostreatus* at-i level with inoculum dose of *Trichoderma asperellum* on-j level.

ε_{ijk}: The random component of the error associated with treatment ij in-k replication.

Table 2: Physical quality of cassava stem fermented by *Pleurotus ostreatus* and *Trichoderma asperellum*.

Variables	*Pleurotus ostreatus* (Po)	*Trichoderma asperellum* (Ta)			Mean	sem	p-values		
		Ta_0	$Ta_{1.5}$	Ta_3			Po	Ta	Po*Ta
Odor	Po_0	3.43	3.17	3.49	3.33^{a}				
	Po_3	3.60	3.70	3.63	3.64^{ab}	0.08	<0.05	0.418	0.507
	Po_6	4.07	3.80	3.60	3.83^{b}				
	mean	3.70	3.56	3.56					
Texture	Po_0	4.53	4.50	4,50	4.51^{b}				
	Po_3	4.43	4.07	3.73	4.08^{b}	0.09	<0.01	0.416	0.938
	Po_6	3.63	3.70	3.83	3.72^{a}				
	mean	4.20	4.09	4.02					
Color	Po_0	3.53^{B}	3.63^{B}	3.60^{B}	3.59^{b}				
	Po_3	3.70^{B}	3.87^{B}	3.73^{B}	3.77^{b}	0.08	<0.01	<0.05	<0.05
	Po_6	4.50^{B}	3.70^{B}	2.67^{A}	3.29^{a}				
	mean	3.58^{ab}	3.73^{b}	3.33^{a}					
Mucus	Po_0	5.00	4.93	4.97	4.97^{b}				
	Po_3	4.93	4.80	4.67	4.80^{ab}	0.06	<0.05	0.249	1.277
	Po_6	4.30	4.73	4.60	4.54^{a}				
	mean	4.74	4.82	4.74					
Other fungi	Po_0	4.87	3.57	4.33	4.26^{b}				
	Po_3	4.23	3.50	3.43	3.72^{a}	0.11	<0.01	<0.01	2.571
	Po_6	3.53	3.53	3.70	3.59^{a}				
	mean	4.21^{b}	3.53^{a}	3.82^{ab}					

$^{a, ab, b}$: Superscripts within the same column show different significant (p<0.05) due to *Pleurotus ostreatus*, Superscripts within the same row show different significant (p<0.05) due to *Trichoderma asperellum*. $^{A, B}$: Superscripts in the same column and row show significant interactions (p<0.05) due to the combination of fungi.

RESULTS AND DISCUSSION

Physical Properties of Cassava Stem Fermented by *Pleurotus ostreatus* and *Trichoderma asperellum* Inoculum

Combination fermentation using *Pleurotus ostreatus* and *Trichoderma asperellum* inocula showed interactions (p<0,05) on the color variable. Data on the physical quality of fermented cassava stems are shown in Table 2.

Table 2 indicates that the combination of *Pleurotus ostreatus* and *Trichoderma asperellum* showed no significant interaction (p>0.05) on the odor of fermented cassava stems. The combination of *Pleurotus ostreatus* 6% and without *Trichoderma asperellum* recorded the highest value (4.07), suggesting the initial stage of the fermentation process. Conversely, the combination *Trichoderma asperellum* 1,5% and without *Pleurotus ostreatus* yielded the lowest value (3.17) compared to other treatments, indicating the presence of a distinctive odor like wood. The two fungi cannot work together because *Trichoderma asperellum* has antagonistic mechanisms, including competition, microparasites, and antibiosis mechanisms. The growth of fungi dominated by *Trichoderma asperellum* can constrain the growth of other fungi such as *Beauveria bassiana*, *Metarhizium flavoviride*, and *Purpureocillium lilacinum* up to 100% (Mu'arif et al., 2024). Fermentation with the addition of *Pleurotus ostreatus* produces a slightly sour odor. Sánchez-García *et al.* (2024) explain that the use of *Pleurotus ostreatus* as a fermentation starter has a sharper fermentation odor. Incubation of *Pleurotus ostreatus* for 40 days can enhance mycelial development, optimizing the fermentation process. The combination of adding these two fungi does not produce an optimal aroma, which is related to the feed ingredient degradation process.

The addition levels of *Pleurotus ostreatus* significantly affected the odor *(p<0.05)*. The addition of *Pleurotus ostreatus* 6% recorded the highest value (3.83), indicating that the cassava stems began to exhibit a distinctive fermentation aroma. This result is higher than the treatment without *Pleurotus ostreatus* and with 3% addition (3.33 and 3.64, respectively), which still retained woody odor. The use of *Pleurotus ostreatus* at a higher level is more effective in intensifying the sour odor, as evidenced by the 6% addition producing a characteristic fermentation aroma. The sour aroma likely signifies effective organic material. Rahmatulwasyiah *et al*. (2024) stated that the use of *Pleurotus ostreatus* which contains phenylalanine and tryptophan (essential amino acids), is capable of producing a distinctive fermented odor. *Trichoderma asperellum* is able to produce the most dominant cellulase enzyme helps break down cellulose, which is the main component of cell walls. *Trichoderma asperellum,* which belongs to *Trichoderma sp*., is a fungal type that produces the most efficient cellulase enzyme for degrading cellulose. The low organic components of cassava stems are also an indication of the small amount of fermentation aroma that appears. The fermentation process produces alcohol through the action of *Trichoderma,* which synthesizes cellulose and hemicellulose, which are used as carbohydrates, thereby influencing the odor of fermentation (Rostini *et al*., 2022).

The combination of *Pleurotus ostreatus* and *Trichoderma asperellum* showed no significant interaction (p>0.05) on the texture of fermented cassava stems. The two fungi possess different functional abilities. Treatment without a combination of the two fungi tended to yield a high value (4.53), which shows that the texture of fermented cassava stems remains in a hard condition with a slight change to be softer. There was a tendency for lower values in the treatment of adding 6% *Pleurotus ostreatus* without *Trichoderma asperellum* (3.63), indicating that the texture of the cassava stem was slightly lumpy and moist. However, statistically, this did not show a significant difference compared to other treatment combinations. Decomposition of the cassava stem substrate occurs as a growing medium for both fungi. The fermentation process enables microorganisms to produce enzymes that decompose hard, complex structures (physically, chemically, and biologically) into simple ones (Rostini *et al*., 2022).

Pleurotus ostreatus is capable to producing lignolytic enzymes, which play a role in the lignin degradation process in lignocellulosic substrates (Dimawarnita and Panji, 2019). Based on statistical analysis, the addition of *Pleurotus ostreatus* showed a very significant effect (p<0.01) on the texture of fermented cassava stems. The 6% *Pleurotus ostreatus* treatment yielded the lowest value (3.72), indicating that the cassava stem had a slightly soft and lumpy texture. Rostini (2017) stated that a successful fermentation process is characterized by a non-clumping texture, a yellowish brown color, and a distinctive fermentation odor.

Based on the observation results, the combination of adding *Pleurotus ostreatus* and *Trichoderma asperellum* to fermented cassava stems resulted in a significant interaction (p<0.01) on the color. The addition of a combination of fungi, 6% *Pleurotus ostreatus* and 3% *Trichoderma asperellum*, produced a fermented cassava stem color with a value of 2.67, which indicates that it produces a dark brown hue. The combination of both fungi at different levels produced colors ranging from brown to light brown. The color changes observed during this fermentation process can also be attributed to biochemical processes such as the degradation of lignocellulose in cassava stems. *Pleurotus ostreatus*, white rot fungus, can degrade lignin by transforming it into phenolic compounds, which impart a brown color. *Trichoderma asperellum* also produces lignolytic enzymes that help accelerate the weathering process. Phenolic compounds such as phenolic acids and tannins are known as the main components of antioxidants found in plants and fungi. In other research, Hatta *et al*. (2014) explained that the addition of a mixture of *Trichoderma veride* and *Pleurotus ostreatus*, regarding inoculum level and incubation time, was influenced by differing growth periods where the vegetative phase of *Pleurotus ostreatus* was relatively longer compared to *Trichoderma veride*.

The combination of *Pleurotus ostreatus* and *Trichoderma asperellum* showed no interaction (p>0.05) with contamination by other fungi. The growth of *Pleurotus ostreatus* fungus is indicated by the presence of white fungus, while the growth of *Trichoderma asperellum* fungus is indicated by the presence of green fungus on the cassava stem media. The combination of adding *Pleurotus ostreatus* 6% and *Trichoderma asperellum* 3% has a color difference, which is also indicated by the presence of a few other fungi that show a slightly black color. Meanwhile, the addition of *Pleurotus ostreatus* 6% displays a good cassava stem color, and the fungi that appear are *Pleurotus ostreatus*. The addition of Trichoderma asperellum 1.5% and 3% indicates the presence of other fungi growth besides *Trichoderma asperellum,* but not *Pleurotus ostreatus*. The emergence of other fungi can be due to contamination of other fungi during the inoculation of *Trichoderma asperellum*, which occurs because the storage of cassava stems is opened. Other fungi that can be found are examined visually by looking for other colors that can be seen.

pH and Temperature of Cassava Stem During Fermentation by *Pleurotus ostreatus* and *Trichoderma asperellum* Inoculum

Combination fermentation using *Pleurotus ostreatus* and *Trichoderma asperellum* inocula showed interactions (p<0,05) on pH variables. Data on the pH and temperature of fermented cassava stems are shown in Table 3.

Based on the observation results (Table 3), the combined addition of *Pleurotus ostreatus* and *Trichoderma asperellum* had a significant interaction (p<0.01) on the pH. This study recorded the highest pH value (7.23) in the combination of *Pleurotus ostreatus* 3% and without the addition of

Table 3: pH and temperature of cassava stem fermented by *Pleurotus ostreatus* and *Trichoderma asperellum*.

Variables	*Pleurotus ostreatus* (Po)	*Trichoderma asperellum* (Ta)			Mean	sem	p-values		
		Ta_0	$Ta_{1.5}$	Ta_3			Po	Ta	Po*Ta
pH	Po_0	4.31^A	7.65^{BC}	8.19^C	6.72				
	Po_3	7.23^B	6.90^B	5.76^A	6.63	0.26	0.734	<0.05	<0.01
	Po_6	5.90^{AB}	6.11^B	7.17^B	6.39				
	mean	5.81^a	6.89^b	7.04^b					
Temperature (°C)	Po_0	28.00	26.33	28.00	27.44^a				
	Po_3	30.00	28.67	29.33	29.33^b	0.35	<0.05	2.884	0.145
	Po_6	30.33	28.33	30.33	29.67^b				
	mean	29.44^{ab}	27.78^a	29.22^a					

$^{a, ab, b}$: Superscripts within the same column show different significant (p<0.05) due to *Pleurotus ostreatus*, Superscripts within the same row show different significant (p<0.05) due to *Trichoderma asperellum*. $^{A, AB, B, BC, C}$: Superscripts in the same column and row show significant interactions (p<0.01) due to the combination of fungi.

Trichoderma asperellum. In contrast, the treatments without the addition of the two fungi produced the lowest pH value (4.31) in fermented cassava stems. The treatment without a starter showed an acidic pH, a condition not optimal for the growth of either *Pleurotus ostreatus* or *Trichoderma asperellum*. Singh *et al.* (2014) also state that the fungus *Trichoderma asperellum* can grow optimally in the pH range of 6.5 - 7.5 towards alkaline pH conditions; even if the pH is higher, it will inhibit the growth of the fungus. This is in accordance with research conducted by Sándor *et al.* (2016) that the optimal pH for the growth of *Pleurotus ostreatus* ranges from 5.5 to 6.5, and mycelial growth occurs in the pH range of 5 to 6.5.

The pH changes can be determined by measuring the cassava stems before and after the fermentation process is complete. After fermentation is complete, Fermentation of cassava stems using 3% and 6% *Pleurotus ostreatus* without *Trichoderma asperellum* showed an improved pH balance. The success of fungal growth in the fermentation process can be determined by knowing the temperature of the fermented material. Based on the pH value of the combination, it shows that the fermentation was successful. This is reinforced by the opinion of Kung *et al.* (2018), who stated that pH is one of the determining factors for the success of fermentation. The resulting pH value shows that the results of cassava stem fermentation are acidic, approaching neutral. If the pH is too acidic, it will stop the growth of *Pleurotus ostreatus* mycelium. Fermentation using *Pleurotus ostreatus* affects the increase in organic matter content, especially protein (Saputra *et al.*, 2024).

The addition of *Pleurotus ostreatus* showed a different significant (p>0.05) with temperature changes during fermentation. The temperature produced in this fermentation process was in the range of 26 °C on the addition of *Trichoderma asperellum* 1.5%, while the addition of *Pleurotus ostreatus* 6% without *Trichoderma asperellum* and also the addition of *Trichoderma asperellum* 3% resulted in a temperature increase of up to 30.33 °C. The temperature is very suitable for the growth of both fungi, and treatment without fungi has a low temperature; the fermentation process is slow, and no fungi grow in the fermented cassava stem. The optimum temperature for fermentation using 3% and 6% fungi is 29.33 °C and 29.67 °C. The temperature in this fermentation process is the ideal temperature for *Pleurotus ostreatus* growth, according to the Ministry of Agriculture (2010), which states that the temperature for *Pleurotus ostreatus* growth is between 28-30 °C, with humidity of 50-60%. Rostini *et al.* (2022) state that every time the fermentation process takes place, the temperature will increase to become hotter, thus having an effect on the structure of the material. Fermentation temperature generally has a close correlation with the pH formed, this is because temperature will affect enzyme activity, microbial metabolism, and the rate of substrate degradation. The optimal temperature for *Pleurotus ostreatus* and *Trichoderma asperellum* growth ranges between 25-30 °C. The fermentation process by microorganisms will increase the temperature, which indicates metabolic activity by the microbes (Mulyono *et al.*, 2021). If the fermentation temperature is low, the degradation process is slower, but it can increase the production of nutritional components due to secondary microbial metabolism, which causes a lower pH after fermentation. Riswandi *et al.* (2017) state that during the process of decomposition of organic materials by microorganisms, bacteria, CO_2 can increase, so that the fermentation temperature can increase.

Chemical Quality of Cassava Stem Fermented by *Pleurotus ostreatus* and *Trichoderma asperellum*

Combination fermentation using *Pleurotus ostreatus* and *Trichoderma asperellum* inocula provides interactions (p<0,05) on crude fiber and nitrogen-free extract variables. Data on the physical quality of fermented cassava stems is shown in Table 4.

Table 4: Nutritional content of cassava stem fermented by *Pleurotus ostreatus* and *Trichoderma asperellum* inoculum.

Parameter	*Pleurotus ostreatus* (Po)	*Trichoderma asperellum* (Ta)			Mean	sem	p-values		
		Ta_0	$Ta_{1.5}$	Ta_3			Po	Ta	Po*Ta
Dry Mater (%)	Po_0	74.74	79.35	79.40	77.83[a]				
	Po_3	82.77	86.70	86.37	85.28[b]	1.08	<0.05	3.017	0.573
	Po_6	88.84	89.21	89.21	89.13[c]				
	Mean	82.12	85.09	85.04					
Organic Mater (% of DM)	Po_0	93.99	93.62	93.68	93.76				
	Po_3	95.37	95.34	95.33	95.35	0.29	3.489	0.077	0.065
	Po_6	93.69	93.37	94.05	93.70				
	Mean	94.35	94.11	94.35					
Crude Protein (% of DM)	Po_0	4.83	5.76	5.27	5.29[a]				
	Po_3	5.97	4.99	4.37	5.11[a]	0.29	<0.05	0.216	1.725
	Po_6	5.53	6.56	7.86	6.65[ab]				
	Mean	5.44	5.77	5.84					
Crude Fiber (% of DM)	Po_0	47.41	40.40	43.26	43.69[ab]				
	Po_3	39.50	38.63	36.12	38.08[a]	0.96	<0.05	2.385	1.126
	Po_6	36.59	33.47	37.47	35.84[a]				
	Mean	41.17	37.50	38.95					
Crude Fat (% of DM)	Po_0	1.05	0.48	0.41	0.65				
	Po_3	0.36	0.35	0.52	0.41	0.06	2.087	0.751	2.649
	Po_6	0.36	0.52	0.47	0.45				
	Mean	0.59	0.45	0.47					
NFE (% of DM)	Po_0	40.19	46.68	44.48	43.78[a]				
	Po_3	49.40	51.26	54.14	51.60[b]	1.01	<0.05	1.722	1.408
	Po_6	51.12	52.69	48.13	50.64[b]				
	Mean	46.90	50.21	48.92					
Total Digestible Nutrient (%)	Po_0	51.44	55.05	53.35	53.28[a]				
	Po_3	57.37	57.42	58.56	57.78[b]	0.56	<0.05	2.203	0.779
	Po_6	57.03	58.85	57.89	57.92[b]				
	Mean	55.28	57.11	56.60					

[a, ab, b]: Superscripts within the same column show different significant ($p<0.05$) due to *Pleurotus ostreatus*; no superscripts within the same row show non-significant ($p>0.05$) due to *Trichoderma asperellum;* No superscripts in the same column and row show non-significant interactions (p<0.05) due to the combination of fungi.

Dry Matter and Organic Matter Content of Fermented Cassava Stem

The addition of *Pleurotus ostreatus* and *Trichoderma asperellum* showed no interaction ($p>0.05$) with to DM and OM content. Treatment without using *Pleurotus ostreatus* and *Trichoderma asperellum* produced the highest DM content of 89.34%, while the treatment without *Pleurotus ostreatus* combined with *Trichoderma asperellum*1.5% recorded able to produce the lowest DM content of 79.35%. This result suggested that the presence of *Trichoderma asperellum* in fermentation has a role in reducing the DM, although its effect is not always consistent in each treatment. However, fermentation can have an effect on DM, such as cassava stem fermentation technology using different levels of urea, which has an effect on DM content (da Silva *et al*., 2024). Dry matter is an important parameter in assessing fermented feed quality, as it affects storage stability and feed utilization efficiency in animals. Variations in the DM content of feed ingredients during fermentation can occur due to several factors, including fungal activity. *Pleurotus ostreatus* stored for 40 days showed a decrease in DM content due to extended storage time, which is by Azzahra *et al*. (2022) who reported that 60-day fermentation with *Pleurotus ostreatus* reduces the DM in empty oil palm bunches by 1.96% and in sawdust by 0.38%. However, no DM decrease was observed during 30 days of storage.

Fermentation with *Pleurotus ostreatus* showed a significant effect ($p<0.05$) on the DM content. Treatment without fungi *Pleurotus ostreatus* has the lowest DM content compared to other treatments, with an average of 77.70%, while the addition of *Pleurotus ostreatus* is able to increase DM to the point addition of 6% DM increased by an average of 89.13%. The increase in DM content is possibly due to the water loss during the fermentation process. This result is different from the research of Suciyanti *et al.* (2015), that the addition of 15 g *P. ostreatus* incubated for 6 weeks reduced the DM content to 88.84% and OM to 77.85% in fermentation durian skin. Feed materials with high moisture content, such as forages, experience an increase in DM content due to water reduction during fermentation (Hilakore *et al.*, 2022). The decrease in DM content in the fermentation process is due to the large amount of substrate used during fermentation. Azzahra *et al.* (2022) noted that the more substrate is broken down, the more water is formed, and as a result, the DM decreases. Treatment without *Trichoderma asperellum* had the lowest average DM content compared to other treatments, namely an average of 82.12%, while the additional treatment *Trichoderma asperellum* 1.5% and 3% experienced an increase in DM content, respectively, 85.09% and 85.04%.

The inclusion of *Trichoderma asperellum* at 0%, 1.5%, and 3% levels resulted in similar OM with values ranging from 94.11% to 94.45%. This indicates minimal changes in OM content after fermentation with *Trichoderma asperellum*. Fermentation provides benefits to improve the quality of the substrate, even though it does not directly change the OM content, but it can change its properties (Samuels *et al.*, 1999). Fermentation on the substrate leads to the conversion of OM into *Pleurotus ostreatus* fungal mycelium, H_2O and CO_2, thereby changing the substrate biomass. The addition of *Pleurotus ostreatus* to empty oil palm bunches for 60 days reduced OM to 90.41% and sawdust to 87.89% (Azzahra *et al.*, 2022).

Crude Protein, Crude Fiber and Fat Content

Based on statistical analysis, the combination of *Pleurotus ostreatus* and *Trichoderma asperellum* showed no significant interaction (p>0.05) on the CP content of fermented cassava stems. The combination of adding 6% *Pleurotus ostreatus* and 3% *Trichoderma asperellum* tends to produce the highest CP content (7.86%). The increase in CP content is possible due to fungal activity, degrading fiber components so that the core of the cell can be digested. Degradation of fiber components in the combination of *Pleurotus ostreatus* and *Trichoderma asperellum* is able to release lignin, cellulose, and hemicellulose bonds in the membrane cell, so that the nucleus containing organic material and crude protein can be utilized. Hatta *et al.* (2014) stated that adding *Pleurotus ostreatus* and *Trichoderma viridae* inocula significantly increased the CP content in copra meal. The lowest CP content in fermented cassava stems was found in the treatment without either fungus (4.83%). This might be attributed to the high crude fiber content in cassava stems and the lack of cell wall degradation, preventing the utilization of the nucleus components.

Increasing the level of *Pleurotus ostreatus* showed a significant effect (p<0.05) on CP content; there was an increase in fermented cassava stem without *Pleurotus ostreatus* (5,03%), and with *Pleurotus ostreatus* 6% CP content increased (6.65%). The increase in pH value of cassava stems after the fermentation process indicates an increasing CP content. The increased CP content after fermentation includes not only protein from cassava stems, but also potentially fungal protein. The post-fermentation CP includes fungal protein, as well as extracellular proteins produced by fungi such as lignocellulolytic enzymes, and some nitrogen-producing metabolites (Zhu *et al.*, 2015). Increasing fungal biomass can contribute to higher CP content in fermented cassava stems because fungi produce enzymes, which are proteins. Based on statistical analysis, the treatment of adding *Trichoderma asperellum* showed no significant effect (p>0.05) on CP content. In accordance with research conducted by Rostini *et al.* (2022), the use of *Trichoderma sp*. incubated for 10 days resulted in high CP content. There was a decrease in the use of *Trichoderma asperellum*, but not significant, possibly because some nitrogen-containing metabolites evaporate (Zheng *et al.*, 2021), so that the CP content increased but was not significantly different, leading to a non-significant increase in CP content.

The Combination of *Pleurotus ostreatus* and *Trichoderma asperellum* showed no interaction (p>0.05) with the CF content of fermented cassava stems. Fermentation without inoculation, *Pleurotus ostreatus* and *Trichoderma asperellum* have the highest average CF content (47.41%), but the treatment of adding *Pleurotus ostreatus* 6% and *Trichoderma asperellum* 1.5%, the CF content decreased to 33.47%. The stem is a high CF component of the cassava plants, which requires feed processing technology to enhance its utility. Hatta *et al.* (2014) explain that the use of a combination of *Pleurotus ostreatus* and *Trichoderma veridae* is able to reduce the crude fiber content of copra cake from 14.88% without mushrooms to 13.94%. Fermentation using *Pleurotus ostreatus* showed a significant (p<0.05) effectively reduced the CF content. According to Palangi *et al.* (2022), there was a significant decrease in the CF content in the substrate grown by *Pleurotus ostreatus*. The substrate that was not grown by fungus had a CF content of 13.33% and decreased to 8.26% when using fungus. Fermentation of cassava stems using *Pleurotus ostreatus* involves enzymatic and non-enzymatic mechanisms, utilizing extracellular enzymes such as lignin peroxidase, manganese peroxidase, and laccase to degrade lignin, and cellulase enzymes to degrade cellulose.

The addition of *Trichoderma asperellum* inoculation resulted in no significant (p>0.05) decrease in the CF content of fermented cassava stems. Treatment without adding *Trichoderma asperellum* up to 3% resulted in a decrease in CF content from 41.17% to 38.39%. The extended fermentation time in fermented cassava stems using this type of *Trichoderma asperellum* is able to reduce CF content because *Trichoderma asperellum* produces cellulose, which breaks down cellulose and hemicellulose into two glucose groups (galactose and arabinose). Rostini *et al.* (2022) found that the addition of 3% addition of *Trichoderma sp* to rumen contents and fermented rice bran for 10 days was able to reduce the CF content from 28.49% without *Trichoderma sp* to 25.73%. A decrease in CF levels due to the delignification process is an indicator of successful fermentation. The addition of *Trichoderma asperellum* shows a decrease in CF content during the fermentation process, which is thought to be caused by *Trichoderma asperellum* and microorganisms found in materials that produce the sulfatase enzyme. The results of this research on cassava stem fermentation are able to reduce CF and can be used as a feed ingredient in complete feed for ruminant livestock to observe consumption and digestibility.

Based on statistical analysis data, the combined treatment of *Pleurotus ostreatus* and *Trichoderma asperellum* showed no significant interaction (p>0.05) on fat. The combination of 3% *Pleurotus ostreatus* and 1.5% *Trichoderma asperellum* produced the lowest fat content (0.35%), while the highest fat content (1.05%) was in the treatment of fermented cassava stems without using fungi. Cassava stems are plant components that have low crude fat content. *Pleurotus ostreatus* also *Trichoderma asperellum,* without combination, showed no significant effect (p>0.05) on the fat. Fat content across different *Pleurotus ostreatus* addition levels was almost similar, but there was a potential decrease in fat content (0.65%) in the treatment without *Pleurotus ostreatus*, and there was a tendency for a decrease with 6% *Pleurotus ostreatus* (0.45%). The decrease in fat content is attributed to the lipase enzyme produced during fermentation with *Pleurotus ostreatus*, which can degrade fat by breaking down triglycerides into fatty acids or glycerol, making fat more readily degradable by *Pleurotus ostreatus*. The addition of *Trichoderma asperellum* showed no significant (p>0.05) effect on the fat. The addition of *Trichoderma asperellum* results in a decrease in fat content, specifically in cassava stems without *Trichoderma asperellum* (0.59%) and a decreasing tendency with 3% *Trichoderma asperellum* (0.47%).

Nitrogen-Free Extract and Total Digestible Nutrient Fermented Cassava Stem

The combination of *Pleurotus ostreatus* and *Trichoderma asperellum* showed no significant interaction (p>0.05) on NFE content. The combination treatment of 3% *Pleurotus ostreatus* and 3% *Trichoderma asperellum* tended to produce the highest NFE content (54.14%), while the combination 3% *Trichoderma asperellum* without *Pleurotus ostreatus* tended to reduce the NFE content to the lowest (44.84%). The high NFE value is due to the addition of the two fungi in the fermentation being able to degrade the fermented cassava stem fiber bonds, so that the cellulose and hemicellulose components of cassava stems can be used as carbohydrates. The NFE consists of carbohydrates, sugar, and starch, which are easily digested by animals; meanwhile, crude fiber is more difficult to digest. According to Lazarus *et al.* (2023), soluble carbohydrates were the main source of substrate needed for the fermentation process. The cassava stems contain carbohydrates that are not easily soluble and need to be optimized through fungal fermentation. The NFE content indicates the carbohydrate value, which ruminant livestock can utilize from forage (Aling *et al.*, 2020).

Treatment with the addition of *Pleurotus ostreatus* showed a significant effect (p<0,05) on NFE of fermented cassava stems. The addition treatment *Pleurotus ostreatus* was able to increase the content of NFE, treatment without *Pleurotus ostreatus* produced the highest NFE content (47.31%), while the addition of *Pleurotus ostreatus* levels of 3% and 6% increased the NFE content consecutively (51.63% and 49.73%). Increased NFE content of *Pleurotus ostreatus* can aid effective OM degradation. Noferdiman *et al.* (2014) stated that fermentation using *Pleurotus ostreatus* can increase the NFE content in feed ingredients, because this fungus helps break down crude fiber into more easily digestible substances. The addition of *Trichoderma asperellum* showed no significant effect (p>0.05) on NFE content. However, the addition of *Trichoderma asperellum* has the potential to increase the NFE content of fermented cassava stems.

Based on the observation results, the combination of *Pleurotus ostreatus* and *Trichoderma asperellum* showed no significant interaction (p>0.05) on TDN content. The treatment with 6% *Pleurotus ostreatus* and 1.5% *Trichoderma asperellum* tended to have the highest TDN content (58.85%), while 3% *Trichoderma asperellum* without *Pleurotus ostreatus* produced the lowest TDN content (53.72%). This combination creates a synergistic effect that optimizes feed material degradation, increases digestible nutrient levels, and raises TDN values. The addition of *Pleurotus ostreatus* showed a significant effect (p<0.05) on TDN content. *Pleurotus ostreatus* is more effective at degrading lignin, which helps break down the cell wall structure and facilitates cellulolytic enzymes like cellulase (produced by *Trichoderma asperellum*). The additional *Trichoderma asperellum* showed no significant (p>0.05) to TDN values. The efficiency of substrate fermentation with a single species is limited due to cell growth constraints and lignocellulose degradation ability. Therefore, using specific microbial consortia consisting of several species is preferred for fermentation, making it an effective strategy for biotransformation (Liu *et al.*, 2025). The TDN content reflects the total energy derived from feed consumed by livestock. Using fungus as an effective fermentation starter can increase TDN. The TDN content in total mix ration containing fermented oil palm leaf stalks using *Aspergillus niger* increased TDN content by 58.20% (Mastopan *et al*., 2014).

The Cost-Benefit

The results found from this study, the use of cassava stems can be used as an effort to handle cassava agricultural waste. Fermentation technology is easy and is commonly carried out by farmers in the South Lampung Regency area.

Hygiene to Control Contamination

To start the inoculation of both fungi, the cassava stems undergo sterilization by steaming. The fungi were then carefully inoculated into the cassava stems using alcohol-sterilized gloves and stored in a sterile room to prevent contamination.

CONCLUSIONS AND RECOMMENDATIONS

The combination of *Pleurotus ostreatus* and *Trichoderma asperellum* improved the color and pH. The addition of *Pleurotus ostreatus* increased crude protein and reduced crude fiber on fermented cassava stems. The addition of *Pleurotus ostreatus* was more optimal than a combination with *Trichoderma asperellum* for cassava stem fermentation. The farmers can easily apply this fermentation method, and the fungi are easy to find in nature. From the research results obtained, it is recommended to use a dose of 6% for *Pleurotus ostreatus* to ferment cassava stems. These results require further research; the next step will be to experiment with the biological quality, such as *in vitro* or *in vivo* digestibility in livestock.

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NOVELTY STATEMENTS

Cassava stems, characterized by a hard texture and high fiber content, can serve as an alternative feed for ruminant livestock through fermentation technology using the fungi *Pleurotus ostreatus* and *Trichoderma asperellum*. Previous research primarily utilized only *Pleurotus ostreatus* on fermented cassava stems.

AUTHOR'S CONTRIBUTIONS

Kusmartono: Make conceptualization and methodology.
Agung Kusuma Wijaya: Analyzed the data, wrote the original draft manuscript, and obtained data resources.
Hartutik: Make formal analysis and writing- review and editing the manuscript.
Mashudi: Make a discussion and investigation.

All authors have declared that they have reviewed and approved the final draft for publication consideration.

Conflict of Interest

The authors declare no conflict of interest.

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