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Physical Quality and Number of Spores from Coffee Pulp Fermented with *Trichoderma viride* Based on The Number of Different Spores

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ABSTRACT

Using coffee pulp as an alternative feed for ruminant livestock is one solution that can be used to overcome the problem of fluctuating green feed availability. The primary and sustainable goal to be achieved is the intensification and expansion of plantation waste based on Coffee pulp as an alternative feed to increase the utility of Coffee pulp. The specific target to be achieved is to determine the ability of *T.viride* with various concentrations to improve physical quality and the ability of molds to maintain their population so that they can produce good quality animal feed with good physical condition to support livestock productivity and growth. This study used a Completely Randomized Design (CRD) consisting of 4 treatments with four replications. The treatment consisted of 0 spores/ml, 10^5 spores/ml, 10^6 spores/ml, and 10^7 spores/ml of *T. viride* addition. The parameters observed were physical quality (color, texture, odor), pH, and number of spores. Based on the research that has been conducted, it can be concluded that the best treatment of *T. viride* mold to support a good coffee pulp fermentation process is at a dose of 10^7 spores/ml of water.

Keywords: coffee pulp, physical quality, pH, spores, *T. viride*

INTRODUCTION

Intensification and expansion of plantation waste is a potential effort to overcome the crisis of ruminant livestock feed in the future. Plantation waste that has the potential to be used as ruminant livestock feed is coffee pulp. Indonesia is one of the world's top coffee producers, with yearly production ranging from 700,000 to 750,000 tonnes and waste estimated at 280,000 to 420,000 tonnes yearly. The wet method of coffee processing is predicted to yield 0.4-0.6 kilograms of wet coffee skin for every kilogram of coffee beans. According to Dinas Pertanian dan Perkebunan Aceh (2021), the coffee skin waste produced in Aceh can reach 30,000 tonnes annually. Coffee pulp is still underutilized as livestock feed because this waste is mainly intended as compost, and some of it is just thrown away. Utilization of alternative ruminant livestock feed based on coffee pulp is a solution that can be done to increase the utility value of plantation waste and overcome the problem of feed availability in the future.

The by-products of coffee fruit peeling consist of 40-45% coffee beans (green beans) and 55-60% coffee pulp (Romadhona et al., 2022). According to research conducted by Pratama et al., (2018), unfermented coffee pulp from Aceh Province contains 8.58% crude protein and 14.54% crude fiber with low digestibility, namely the dry matter digestibility coefficient (IVDMD) 46.89% and the organic matter digestibility coefficient (IVOMD) 42.27%. Low crude protein and high crude fiber values are inhibiting factors in the use of coffee pulp as animal feed. In addition, organoleptic tests on the physical quality of coffee pulp fermentation must be carried out because they are the initial step in determining the quality of good fermentation results based on color, aroma, and texture. If the physical quality is good, the coffee pulp fermentation research can be continued with a proximate test to determine the nutritional quality of the coffee pulp fermentation results. Efforts can be made to improve the physical quality and fermentation quality of coffee pulp by using fermentation technology. Fermentation is an effort that can be done by utilizing microbes and biological agents to improve the quality of animal feed ingredients. (Samadi et al., 2016).

Efforts to improve coffee pulp's physical quality and fermentation quality are carried out by applying *Trichoderma viride* mold. *T. viride* is a mold that produces cellulase enzymes with a cellulase index of 3.38, so it is widely applied in biotechnology (Gupta et al., 2014). The use of *T. viride* mold in the fermentation process is a

cheap, easy, and efficient technology to improve the quality of coffee pulp to increase the utility value of plantation waste and extend shelf life. This study aimed to determine the effect of using *T. viride* on coffee pulp with different doses on the physical quality, pH value, and number of *T. viride* spores after fermentation.

MATERIALS AND METHODS

Research Material

The 60 kg of coffee pulp used came from coffee farmers in Central Aceh Regency. The *T. viride* starter was obtained from the Plant Disease Laboratory of Syiah Kuala University. Rice flour, molasses, and urea were purchased from agricultural stores in Banda Aceh city.

Research Procedure

A total of 16 samples of fermented coffee pulp with different numbers of spores were used in this study. Before being fermented, the coffee pulp was dried for 5 days under direct sunlight and then ground using a cutting mill machine to reduce the particles. All ground coffee pulp was homogenized with 3% rice flour from the weight of the substrate. In the first stage, 3% molasses and 1% urea were dissolved in water with a weight of 60% of the substrate, then *T. viride* was added as much as 10^5 spores/ml, 10^6 spores/ml, and 107 spores/ml, while in the control treatment, no *T. viride* was added. After being homogeneous, the solution was mixed into the coffee pulp with a weight of 40% of the ground substrate and then stirred again until all the ingredients were mixed homogeneously. All samples were put into plastic silos according to their respective treatments and stored at room temperature for aerobic fermentation (plastic silos were pierced with the same distance and number) for 21 days. Each treatment consisted of 4 replications. After harvest, all samples were dried in an oven at 60°C for two days.

pH Value Determination

The substance to be quantified with 100 grams of pH is measured and placed into an Erlenmeyer flask, followed by 100 ml of distilled water, then vortexed until homogeneous. Subsequently, immerse for 15 minutes. The medium is assessed with a pH meter. The output from the pH meter represents the pH value of the sample.

Organoleptic Test

Physical quality measurements include color, texture, and odor. Physical quality assessment involves 20 non-standard panelists (people not trained in conducting organoleptic/sensory assessments and tests). The assessment method uses a scoring method based on tests conducted by Daning and Karunia, (2018) and Basri et al., (2019) as follows:

Table 1. Physical Quality Parameter

Value	Color[1]	Texture[2]	Odor[1]
1	Light brown	Solid	Fresh Sour
2	Dark brown	Mushy	Sour
3	Black	Mushy and Slimy	Rotten

Source : Daning and Karunia, (2018)[1]; Basri et al., (2019)[2]

In color testing, the light brown category is in the range of 1-1.75; the dark brown category is in the range of 1.76-2.5; and the black category is in the range of 2.6-3.35. In texture testing, the solid category is in the range of 1-1.75; the mushy category is in the range of 1.76-2.5; and the mushy and slimy category is in the range of 2.6-3.35. In odor testing, the fresh sour category is in the range of 1-1.75, the sour category is in the range of 1.76-2.5, and the rotten category is in the range of 2.6-3.35.

Number of Spores

To count spores, a suspension or dilution is created. One gram of Trichoderma starter, grown in maize medium, is combined with nine milliliters of distilled water that has been sterilized. The mixture is then vortexed until the fungal spores are extracted from the medium. Then, transfer up to 1 milliliter of spores into a second test tube filled with 9 milliliters of distilled water. Repeat the homogenization process until the fourth dilution. One drop of spore suspension is placed on the hemocytometer, which is covered with a cover glass to complete the counting process and then examined at 100x magnification using a microscope. Once you've located the initial box, magnify it again with 400x magnification until

you find a 1 mm box in the center. The spores are ready to be counted after locating a 1 mm box and identifying five counting points—four in each corner and one in the center.

The number of spores is calculated based on the following formula:

$$\mathbf{K} = 1 + \frac{\mathrm{t.\,d}}{\mathrm{N.\,0.25}} x\ 10^6$$

Information:
K : Number of spores
t : Number of spores in all sample boxes
d : Dilution Factor
n : The sum of all sample boxes counted
0.25 : Standard size of hemocytometer (mm)

Data Analysis

The design used in this study was a Completely Randomized Design (CRD) with four treatments and four replications using *T. viride* mold as a treatment and incubated for 21 days; the treatment of this study was without *T. viride, T. viride* 10^5 spores/ml, *T. viride* 10^6 spores/ml and *T. viride* 10^7 spores/ml. Data were analyzed statistically by analysis of variance using SPSS software (version 16 for Windows), with the following mathematical model:

$$Yij = \mu i + \tau i + \varepsilon ij$$

If differences are found between treatments, the analysis is continued with Duncan's multiple range test (DMRT) (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

Physical Quality of Fermented Coffee Pulp

The physical quality of fermented coffee pulp was evaluated organoleptically by examining its color, odor, and texture. The evaluation outcomes are displayed in Table 2.

Table 2. Physical quality of fermented coffee pulp with T. *viride*

Treatment	Color	Odor	Texture
P0	1.75 ± 0.50	2.50 ± 0.57	2.00 ± 0.81
P1	2.25 ± 0.50	2.25 ± 0.57	2.00 ± 0.81
P2	2.00 ± 0.81	2.00 ± 0.82	1.50 ± 0.57
P3	1.25 ± 0.50	1.25 ± 0.50	1.25 ± 0.50

P0: without *T.viride*; P1: *T.viride* 10^5 spores/ml; P2: *T.viride* 10^6 spores/ml; P3: *T.viride* 10^7 spores/ml

Color

Color is an indicator of the quality of fermentation. The material's original color indicates good quality, while other colors indicate medium to low quality. Based on the results of the analysis in Table 2, it can be seen that the color of the coffee pulp fermented using *T. viride* was not significantly different (P>0.05), with a range of values from 1.25 to 2.25. The best results were found in P3 (T.viride 10^7 spores/ml) with a value of 1.25 (light brown category). The results of this study are from the survey conducted by Basri et al., (2019), which stated that coffee pulp fermented using *T. viride* mold produces a light brown color, but different from the study conducted by Daning and Karunia, (2018) Which stated that coffee pulp fermented with *T. viride* will produce a brownish-yellow color. The color changes that occur are caused by the aerobic respiration process as long as the oxygen supply is still available until the carbohydrates contained in the material run out. Carbohydrates will be easily oxidized into CO2 and water, which, with heat's emergence, causes the temperature to rise. (Banožić et al., 2020).

Odor

Besides color, another signal is odor. The odor of fermentation influences physical quality, with favorable coloration correlating to a pleasant smell. Based on the results of the analysis in Table 2, it can be seen that the odor of the coffee pulp fermented using *T. viride* was not significantly different (P>0.05), with a range of values from 1.25 to 2.50. The best results were found in P3 (*T. viride* 10^7 spores/ml) with a 1.25 (fresh sour category) value. The study's findings are consistent with Daning and Karunia, (2018) Fresh sour odor, which contrasts with Basri et al., (2019) Sour odor. Due to the fermentation process, our study's results have a unique odor. Volatile fatty acids are the end product of an aerobic reaction during fermentation and are formed on days 1 through 21. Furthermore, ideal

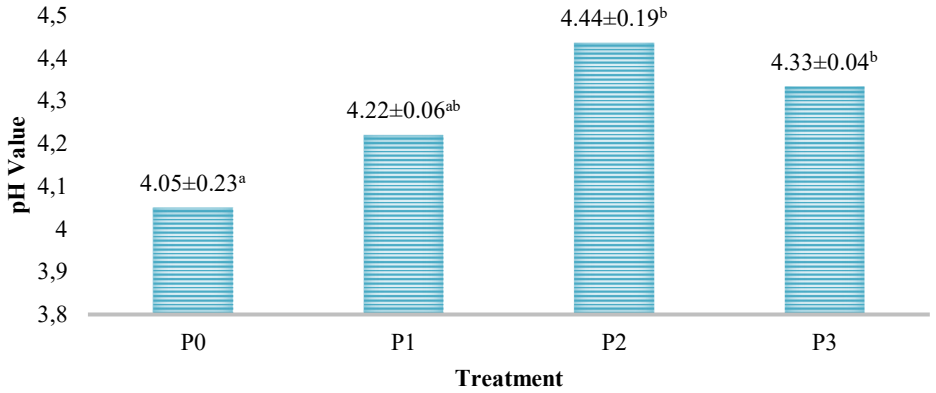
fermentation conditions demonstrate *T. viride* efficacy.

Texture

The feel of a material's surface that is purposefully designed to elicit a positive or negative quality response is represented by its texture. Based on the results of the analysis in Table 2, it can be seen that the texture of the coffee pulp fermented using *T. viride* was not significantly different (P>0.05), with a range of values from 1.25 – 2.0. The best results were found in P3 (*T. viride* 107 spores/ml) with a 1.25 (solid category) value. The texture of the fermented coffee pulp is neither slimy nor soft; instead, it is dry with slight moisture, resulting in an aesthetically pleasing appearance. The fermentation of feed utilizing *T. viride* can decompose the cellulose component in coffee pulp due to the secretion of the cellulase enzyme during the fermentation process (Kodri et al., 2013). This study aligns with the findings of Christi et al., (2018) which indicated that concentrate fermented with Saccharomyces cerevisiae and EM4 yields a somewhat dry mouthfeel. Lang et al., (1997) asserts that the fermentation process yields varying textures based on the material utilized. The texture of the fermented product, regardless of its dryness, is contingent upon the material's water content. A reduced water concentration in the material results in a fermentation product with a drier texture, whereas an elevated water content yields a texture that ranges from slightly moist to wet (Telew et al., 2017).

pH Value of Fermented Coffee Pulp

The presence of pH is related to the activity of enzymes produced during mold growth. The average pH value of fermented coffee pulp utilizing *T. viride* at various dosages is illustrated in the subsequent figure:



P0: without *T. viride* ; P1: *T.viride* 10^5 spores/ml; P2: *T.viride* 10^6 spores/ml; P3: *T.viride* 10^7 spores/ml

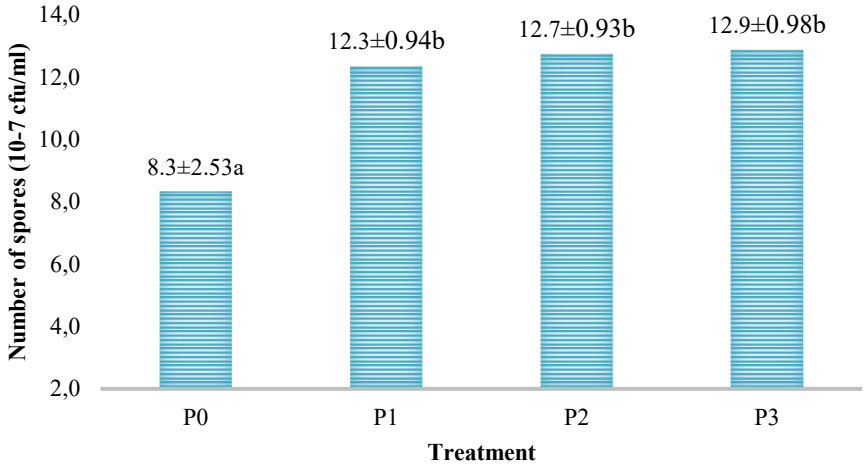
Figure 1. pH value of fermented coffee pulp using *T. viride* with different doses. Numeric notations followed by different superscripts on the diagram indicate a significant effect (P>0.05).

Based on the results of the variance analysis, it was shown that the treatment had a significant effect (P>0.05) on the pH value of fermented coffee pulp with a pH range of 4.05 – 4.44. Duncan's multiple area test indicated no significant difference between P0 and P1 but revealed substantial differences between P0 and P2 and P0 and P3. This indicates that the *T. viride* mold can proliferate on coffee pulp and decompose the fibrous material into lactic acid. Kredics et al., (2003) assert that the optimal pH range for *Trichoderma sp.* growth is between 2 and 6.

Figure 1 illustrates a rise in pH from treatments P0 to P2 and a decline in P3. The dosage of spores administered is believed to influence the activity of microorganisms during fermentation. The findings of this study align with the research by (Winanda et al., 2023), which indicated a reduced pH in silage due to elevated microbial activity. This is in line with Abedi and Hashemi, (2020), who stated that lactic acid microorganisms are a group of microorganisms that usually convert carbohydrates into lactic acid. The pH value in this research is classified as "good," specifically within the range of 4.05 to 4.44. This is consistent with the position of (Kurniawan et al., 2016), who asserted that the quality of agricultural waste fermentation is influenced by the degree of acidity, as a suitable pH ranges from 4.2 to 4.5. High pH (> 4.8) and low pH (<4.1) are indicative of low-quality fermentation.

Number of Spores of Fermented Coffee Pulp

The quantification of spores serves as an indicator to assess the growth potential of T. viride mold on a specific substrate. The average number of spores of fermented coffee pulp utilizing *T. viride* at various dosages is illustrated in the subsequent figure:



P0: without *T.viride*; P1: *T.viride* 10^5 spores/ml; P2: *T.viride* 10^6 spores/ml; P3: *T.viride* 10^7 spores/ml

Figure 2. Number of spores of fermented coffee pulp using *T. viride* with different doses. Numeric notations followed by different superscripts on the diagram indicate a significant effect (P>0.01).

Based on the results of the analysis of variance, it was shown that the treatment had a very significant effect (P>0.01) on the number of spores of fermented coffee pulp with a spores range of 8.3 x 10^7 cfu/ml – 12.9 x 10^7 cfu/ml. The results of Duncan's multiple range test showed that the P0 treatment was significantly different from the P1, P2, and P3 treatments, and there was no difference between the P1, P2, and P3 treatments. Figure 2 illustrates that *T. viride* may be cultivated across all treatments, although exhibits varying spore count outcomes. The best number of spores was found in the P4 treatment because the P4 treatment gave the most spores compared to other treatments. This indicated that adding the number of *T. viride* spores of 10^7 spores/ml of water would increase the growth power of *T. viride* on the coffee pulp substrate.

An increased number of spores facilitates optimal mold growth, as a greater quantity of agents can effectively exploit the nutrients in the medium. Minimal fungal supplementation leads to reduced pure protein concentration. This results from the restricted potential for microbial growth and reproduction, leading to suboptimal enzyme synthesis as a metabolic byproduct (Purwati and Windyasmara, 2019). Similarly, an excessively high inoculum dose reduces nutrients, resulting in suboptimal fermentation conditions. Mutlu et al., (2020) asserted that an excessive number of microorganisms can lead to accelerated sporulation, hence diverting energy away from cellular multiplication, whereas a limited microbial population results in inadequate growth. Wang and Wu, (2023) states that organic carbon compounds available to fungi for synthesizing new cellular material include simple molecules like monosaccharides, organic acids, and alcohol-bound sugars, as well as short-chain and long-chain carbon polymers, in addition to complex compounds such as carbohydrates, proteins, lipids, and nucleic acids.

CONCLUSION

Based on the research that has been conducted, it can be concluded that the best treatment of *T. viride* mold to support a good coffee pulp fermentation process is at a dose of 10^7 spores/ml of water. This can be seen from the color, odor, and texture scores falling into the best category and the presence of the most prominent spores in P4 (107 spores/ml water); the large number of spores supports more efficient use of nutrients. In terms of pH value, all treatments are in the range of pH 4, which indicates that all treatments are in acidic conditions.

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