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Effects of solid-state fermentation using *Trichoderma harzianum* and *Saccharomyces cerevisiae* on proximate and fiber content analysis of coffee husk (*Coffea canephora* L.)

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Abstract Solid-state fermented coffee husk (SSFCH) showed a significant effect ($p<0.05$) in terms of crude protein (CP), dry matter (DM), ash, ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) content. Solid state fermented coffee husk with *Trichoderma harzianum* (SSFCH*Th*) exhibited the highest CP content (14.25%) and lowest DM (79.93%), ash (4.98%) across all treatments. SSFCH*Th* reduced fiber content composition for NDF (54.02%) and ADF (44.99%) compared to unfermented coffee husk (UCH) and solid-state fermented coffee husk with *Saccharomyces cerevisiae* SSFCH*Sc*. Therefore, solid-state fermentation with *Trichoderma harzianum* is enhanced by a proximate analysis of coffee husk, which increases its crude protein content while reducing fiber components, making it suitable as animal feed.

Keywords: Animal nutrition, Coffee husk, *Saccharomyces cerevisiae*, Solid-state fermentation, *Trichoderma harzianum*

Introduction

In the Philippines, coffee has an important role in the economy, particularly its agricultural sector, especially in the Caraga region which is known for its high-quality robusta coffee variety. The Philippines ranks as the second-largest consumer of coffee in Southeast Asia (Chengappa and Devika, 2016; Luat *et al.,* 2022) and cultivates four varieties of coffee: arabica, robusta, excelsa, and liberica. The booming number of coffee shops both local and international highlights the growing demand for coffee in the market. According to Tan (2021), coffee provided businesses and income to many local farmers. Consequently, coffee production poses a significant amount of waste such as husk, pulp, silver skin, and spent grounds which contributes to environmental pollution. These by-products constitute approximately 50% of the weight of the coffee beans (Gilberto *et al.*, 2020). It is often overlooked for its potential effectiveness as an agricultural input. Feed is important in livestock production, it represents the largest proportion

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of production costs, ranging from 70-80%. Recently, the cost of growing livestock and poultry has been declining due to increased feed costs and competition between crops for food and animal feed. Investigating the viability of agricultural by-products such as coffee husk as valuable feed presents a solution to these challenges. Franca and Oliveira (2009) mentioned coffee by-product utilization as an alternative feed because of its nutrient content, bioactive compounds, and phytochemicals. It can be a source of energy in compounding feed, but it contains a high amount of fiber, making it less digestible to monogastric animals like poultry and swine.

Solid-state fermentation (SSF) is a biological method that focuses on the cultivation of microorganisms on a substrate with minimal water content. Additionally, it produces enzymes, aromas, organic acids, and protein-enrichment (Abu-Yazid *et al.,* 2017). SSF showed potential in enhancing the nutritive value and digestibility of an agro-industrial by-product (Morales et *al.,* 2018). Improving the coffee husk quality by solid-state fermentation will align with the goal of the Philippine National Agriculture and Fisheries Development and Extension Agenda (DA-NAREA) 2023-2028, which is the development of feed processing and techniques to enhance local feed ingredients' digestibility and bioavailability. *Trichoderma harzianum* as a fermentation microorganism has been used on different agricultural wastes (Abo-Siada *et al.,* 2018). It has a cellulose-degrading ability that is effective in degrading the complex fiber content, resulting in a more digestible and nutritionally effective feed ingredient. On the other hand, *Saccharomyces cerevisiae* contributes to the enhancement of by-product substrate nutrient value. Apart from its ability to break down carbohydrates, *Saccharomyces cerevisiae* also reduces the presence of anti-nutritional factors, improves digestibility, synthesizes vitamins and antioxidants, and enhances mineral availability (Hassaan *et al.,* 2015). So far, there is limited literature on solid-state fermented coffee husks particularly robusta variety with *Trichoderma harzianum* and *Saccharomyces cerevisiae*. This study evaluated the proximate analysis and fiber content of solid-state fermented coffee husk.

Materials and methods

Time and place of the study

This study was conducted at the Department of Animal Science (DAS), Animal Nutrition Laboratory at Caraga State University, Butuan City, Agusan del Norte, Philippines.

Preparation of Trichoderma harzianum

The *Trichoderma harzianum* used in the study was pure culture from the Department of Plant and Soil Science Laboratory of Caraga State

University. The strains were obtained from the culture bank of the Philippine National Collection of Microorganisms Laboratory, University of the Philippines, Los Baños. *T. harzianum* was then sub-cultured on potato dextrose agar (PDA) medium and was incubated at 28°C for seven days.

Unfermented coffee husk

The coffee husk (robusta variety) was collected from a hulling facility in Simbalan, Buenavista, Agusan del Norte, Philippines. Samples were sterilized for two hours using an oven at 160°C. It was then milled and sieved through a 1mm sieve to produce unfermented coffee husk powder, and stored in tightly sealed containers.

Solid State Fermentation (SSF) of coffee husk using starters (Trichoderma harzianum and Saccharomyces cerevisiae)

Coffee husks were sterilized before solid-state fermentation. SSF was conducted following the procedure of Khasanah *et al.* (2022) with some modifications. *Trichoderma harzianum* and *Saccharomyces cerevisiae* with 2.5 grams was diluted with six hundred ml of distilled water and molasses (15 ml) (da Silveira *et al.,* 2019). Then it poured into a one-kilogram sterilized coffee husk and was fermented for 14 days (Zepf and Jin, 2013). After solid-state fermentation, it was oven-dried at 60°C for 72 hours and milled and kept in tightly sealed containers.

Experimental Design, Treatments, and Proximate analysis of unfermented and solid-state fermented coffee husk

The experiment was conducted in a completely randomized design with three treatments, having 3 replications. Treatments were as follows: T1: Unfermented Coffee Husk, T2: Solid-state fermented Coffee Husk with *Trichoderma harzianum* (SSFCH*Th*) T3: SSFCH with *Saccharomyces cerevisiae* (SSFCH*Sc*). Samples were submitted to a commercial laboratory to determine proximate analysis following AOAC (1998) methods in dry matter (DM), crude protein (CP), ash, crude fiber (CF), and ether extract (EE). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed according to Goering and Van Soest (1970).

Statistical analysis

Data was analyzed using the general linear model of Statistical Analysis System (SAS) on Demands for Academics software in a completely blocked design. The differences among treatments were determined using Tukey's Honestly Significant Difference (HSD) test at a significant level (p

<0.05). The results was presented as mean values and standard error of the means.

Results

The proximate composition of unfermented coffee husk (UCH) and solid-state fermented coffee husk with *Trichoderma harzianum* (SSFCH*Th*), and *Saccharomyces cerevisiae* (SSFCH*Sc*) was presented in Table 1. Solid-state fermentation significantly affected the dry matter content of UCH, SSFCH*Th*, and SSFCH*Sc* ranging from 79.93% to 83.90%. For crude protein (CP) content, SSFCH*Th* obtained the highest crude protein (CP) followed by SSFCH*Sc* and UCH. The total CP content recorded was 14.25%, 11.88%, and 10.97%, respectively. Concurrently, solid-state fermented coffee husk shows lower ash and ether extract (EE) content compared to unfermented coffee husk. The unfermented coffee husk (6.02%) had the highest ash content, followed by *S. cerevisiae* (5.10%), and *T. harzianum* (4.98%). Meanwhile, significant differences ($p<0.05$) were observed in ether extract (EE). Interestingly, UCH exhibited the highest EE with 3.29% followed by SSFCH*Th* (2.40%) and SSFCH*Sc* with 0.83%.

Table 1. Proximate analysis of unfermented and solid-state fermented coffee husk

Item	Treatment			SEM	*p-value*
	UCH	SSFCHTh	SSFCHSc		
DM%	83.90[a]	79.93[b]	81.67[c]	0.17	<0.0001
CP%	20.97[b]	14.25[a]	11.88[b]	0.04	0.0051
Ash%	6.02[a]	4.98[b]	5.10[b]	0.45	0.0001
EE%	3.29[a]	2.40[a]	0.83[b]	0.36	0.0080

UCH - unfermented coffee husk, SSFCH*Th* - solid-state fermented coffee husk with *Trichoderma harzianum*, SSFCH*Sc* - solid-state fermented coffee husk with *Saccharomyces cerevisiae*, DM - dry matter; CP -crude protein; EE - ether extract; SEM - standard error mean.
Values with different superscripts within a row are significant at ($p < 0.05$).

Shown in the fiber content analysis of unfermented and solid-state fermented coffee husk is shown in Table 2. Solid-state fermentation had no significant effect on the crude fiber (CF) content ($p>0.05$) of coffee husk. Although not significant, solid-state fermented coffee husk with *T. harzianum* (SSFCH*Th)* had the lowest crude fiber (CF) content at 23.84%, followed by solid-state fermented coffee husk with *S. cerevisiae* (SSFCH*Sc*) with 25.48%, and unfermented coffee husk (UCH) with 25.51%. Moreover, NDF and ADF showed significant differences ($p<0.05$). NDF value of the unfermented coffee husk exhibited the highest value of 56.60% compared to solid-state fermented with *S. cerevisiae (55.78%)* and *T. harzianum (54.02%).* Additionally, notable differences ($p<0.01$) were also observed in ADF, the

solid-state fermented coffee husk had reduced ADF value with 44.99% (SSFCH*Th)* and 48.42% (SSFCH*Sc*) compared to unfermented coffee husk with 49.82%.

Table 2. Fiber content analysis of unfermented and fermented coffee husk (FCH)

Item	Treatment			SEM	*p-value*
	UCH	SSFCH*Th*	SSFCH*Sc*		
CF%	25.51[a]	23.84[a]	25.48[a]	0.94	0.4113
NDF	56.60[a]	54.02[ab]	55.78[a]	0.52	0.0319
ADF	49.82[a]	44.99[b]	48.42[a]	0.69	0.0067

UCH - unfermented coffee husk, SSFCH*Th* - solid-state fermented coffee husk with *Trichoderma harzianum*, SSFCH*Sc*- solid-state fermented coffee husk with *Saccharomyces cerevisiae*, CF - crude fiber; NDF- neutral detergent fiber; ADF- acid detergent fiber; SEM-standard error mean

Values with different superscripts within a row are significant at ($P < 0.05$).

Discussion

In this study, the reduction in dry matter (DM) content can be attributed to the metabolic activities of the fungi (*T. harzianum and S. cerevisiae*), which convert complex organic compounds present in the substrate into simpler forms. Supporting this notion, Chiang *et al.* (2010) suggested that the decrease in dry matter content may result from the utilization of carbohydrates by fungi for protein synthesis. Moreover, numerous studies reported similar findings in dry matter reduction using solid-state fermentation, and it was associated with the increase of crude protein content (Chiang *et al.,* 2010; Xu *et al.,* 2012; Hu *et al.,* 2016).

Coffee husk crude protein content ranges from 7-8 percent (Cangussu *et al.* (2021). However, its result contradicted the present study, which was higher than the previous findings. Wang and Daun (2003), mentioned that the type of variety of a certain feedstuff and environmental condition had a significant effect on its protein content. According to Setiyatwan *et al.* (2018), during duckweed fermentation, *T. harzianum* has the highest crude protein value followed by *S. cerevisiae*. This was due to the increased microbial population of fungi because as they grow and proliferate, they contribute to the overall increase in crude protein content of the substrate. The enhancement of CP content was attributed to the increased extracellular protein of the fungi, its production of enzymes, and activities (Liang et *al.,* 2009).

Ash served as a mineral content indicator present within a substrate (Rashad *et al.,* 2011). Additionally, the ash content significantly impacts the determination of NDF and ADF (Ibarruri and Hernández, 2018). Moreover, ash content of coffee husk ranges from 3% to 7%, although it has limited literature on ash content (Cangussu *et al.,* 2021). In the current study, a reduction in crude ash content was observed after fermentation. This finding is closely in line with the results obtained from the previous study by Soedjatmiko *et al.* (2019) mentioned a reduction of ash content because it was utilized by *Trichoderma reesei* for its growth in fermentation. It was very likely that solid-state fermentation had metabolic activities that utilize minerals, thereby reducing ash content (Osman, 2007; Beebe *et al.,* 2000). Furthermore, the higher ash content in unfermented substrates compared to fermented ones indicates a consistent pattern of ash reduction during fermentation across various substrates (Igbabul *et al.,* 2014).

In terms of ether extract (EE) content in coffee husk, UCH attained the highest value with 3.29%. However, the effect of solid-state fermentation with *S. cerevisiae* (0.83%) was more pronounced compared to *T. harzianum* (2.40%). This was supported by Teng *et al.,* (2017), who found a reduction in EE content in solid-state fermented wheat bran with *Saccharomyces cerevisia*e. *S. cerevisiae,* on the other hand makes lipases more rapidly than *T. harzianum*. Moreover, when *Saccharomyces cerevisiae* has insufficient supply of carbon, neutral lipids will be degraded to produce free fatty acids, which are then utilized for energy through β-oxidation or absorbed from the environment (Klug *et al.,* 2014; Wang *et al.,* 2024).

Meanwhile, a slight reduction in crude fiber content of coffee husk indicates the effectiveness of solid-state fermentation. The result of the present study was supported by Setiyatwan *et al.* (2018), who observed a reduction in fiber content in duckweed when fermented with *T. harzianum.* A study cited by Hatta *et al.* (2014) found that *Trichoderma sp.* reduces lignocellulose and produces cellulase enzymes, which break down complex carbohydrates in plant cell walls into simpler sugars, aiding in crude fiber degradation. Moreover, fermenting coffee husk with *Saccharomyces cerevisiae* can degrade starch, dietary fiber, and cyanide and amylase (Nsereko *et al.,* 2002; Oboh and Akindahunsi, 2003; MacLellan *et al.,* 2010). For this reason, *Saccharomyces cerevisiae* does not possess the same cellulolytic capability as *Trichoderma harzianum,* which degrades explicitly lignin. However, it still impacts the crude fiber but is less pronounced than *T. harzianum*.

Neutral detergent fiber (NDF) value assesses cellulose, hemicellulose, and lignin levels in a particular substrate (Sanz-Sáez *et al.,* 2012). Conversely, acid detergent fiber (ADF) represents a plant material portion that is not digestible for animals, particularly its primary constituents, which are cellulose and lignin (MacLellan *et al.,* 2010). The result of this study found that *T. harzianum* was better at breaking down NDF and ADF in solid-

state fermented coffee husk in all treatments. Moreover, Pan *et al.* (2018), mentioned that *T. harzianum* makes more cellulose and xylanase enzymes during fermentation, which break down the cellulose and hemicellulose in rice straw. *T. harzianum* produces cellulose-degrading enzymes, resulting in an over 50% decrease in cellulose content (Halifu *et al.,* 2029). In addition, hemicellulose and cellulose concentrations were reduced using fungi namely *Sclerotium rolfsii, Trichoderma harzianum, Trichoderma longiobrachiatum, Trichoderma koninggi*, and *Aspergillus niger* in palm kernel cake (Iluyemi *et al.,* 2006). On the other hand, *Saccharomyces cerevisiae* in the present study slightly reduced the amount of coffee husk's NDF and ADF content. Because hemicellulose has a complex structure, which *Saccharomyces cerevisiae* cannot degrade completely compared to *Trichoderma reesei* (Tabañag and Tsai, 2018). *Saccharomyces cerevisia*e breaks down fibers and converts them to sugars and starch (Nsereko *et al.,* 2002). Hence, this study shows that *Trichoderma harzianum* effectively improved the quality of coffee husks through solid-state fermentation in terms of dry matter, crude protein, ash, ether extract, neutral detergent fiber, and acid detergent fiber. Moreover, solid-state fermented coffee husk presents an opportunity as an animal feed, thereby reducing waste production and pollution while contributing to suitable animal production and nutrition. Further research is needed to investigate the effects of solid-state fermented coffee husk as an alternative feed for livestock and poultry, such as conducting caffeine analysis, amino acid profiles, digestibility studies, and feeding trials.

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Conflict of interest

The authors declare no conflict of interest.

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