# Fermentation of Cocoa Pods Husk Using Turmeric Powder and Aspergillus niger: Effects on Fiber Composition and Antinutrients

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# ABSTRAK

Atmaja IGM, Ismartoyo, Natsir A, Syahrir S. 2023. Fermentasi kulit buah kakao menggunakan kunyit bubuk dan *Aspergillus niger*: pengaruh terhadap komposisi serat dan anti-nutrisi. JITV 28(4):259-267. DOI:http://dx.doi.org/10.14334/jitv.v28i4.3180.

Pengolahan kulit buah kakao (KBK) secara fermentasi dengan *Aspergillus niger* serta tambahan herbal seperti kunyit, diyakini mampu meningkatkan kualitas bahan pakan asal limbah perkebunan. Tujuan penelitian ini adalah untuk menurunkan kandungan fraksi serat dan antinutrisi KBK melalui proses fermentasi menggunakan *A.niger* dan penambahan kunyit dengan level yang berbeda. Percobaan dilaksanakan berdasarkan rancangan acak lengkap yang terdiri dari delapan perlakuan dan tiga ulangan. Perlakuan terdiri dari: P0: kontrol, P1: fermentasi KBK dengan *A. niger*, P2, P3 dan P4: fermentasi (KBK + 0,5; 1,0 dan 1,5% kunyit bubuk) dengan *A. niger* P5, P6 dan P7: fermentasi KBK dengan *A. niger* + 0.5; 1,0 dan 1,5% kunyit bubuk. Variabel yang diamati adalah komposisi serat dari KBK serta antinutrisi. Hasil penelitian menunjukkan bahwa fermentasi KBK menggunakan *A. niger* dengan kadar bubuk kunyit yang berbeda nyata menurunkan kandungan fraksi serat yaitu; NDF, ADF, dan hemiselulosa dibandingkan dengan KBK yang tidak difermentasi. Kandungan anti-nutrisi, fermentasi menggunakan *A. niger* dengan penambahan 1,5% kunyit sebelum fermentasi. Kesimpulannya, fermentasi KBK menggunakan *A. niger* dengan penambahan 1,5% kunyit sebelum fermentasi menurunkan kandungan serat dan komponen antiutrisi pada KBK.

Kata Kunci; Antinutrisi, Aspergillus niger, Fraksi Serat, Kulit Buah Kakao, Kunyit

### ABSTRACT

Atmaja IGM, Ismartoyo, Natsir A, Syahrir S. 2023. Fermentation of cocoa pods husk using turmeric powder and *Aspergillus niger*: effects on fiber composition and antinutrients. JITV 28(4):259-267. DOI:http://dx.doi.org/10.14334/jitv.v28i4.3180.

Processing of cocoa pods husk (CPH) by fermentation with *Aspergillus niger* (*A. niger*) and additional herbs such as turmeric is believed to be able to improve the quality of feed ingredients from plantation byproducts. The purpose of this study was to evaluate the effectiveness of the fermentation process using *A. niger* and the addition of different levels of turmeric in reducing the fiber fraction and antinutrient contents of CPH. The experiment used a completely randomized design of eight treatments and three replications. The treatments consisted of P0: control; P1: CPH fermented with *A. niger*; P2, P3 and P4: fermented (CPH + 0.5; 1.0 and 1.5% turmeric powder) with *A. niger*; P5, P6 and P7: fermented CPH with *A. niger* + 0.5; 1.0 and 1.5% turmeric powder were the fiber composition of CPH as well as antinutrients. The results showed that CPH fermentation using *A. niger* with different levels of turmeric powder significantly reduced the content of fiber fractions, i.e., NDF, ADF, and hemicellulose, compared to that of unfermented CPH. For antinutritional content, fermentation using *A. niger* with different levels of turmeric decrease the lignin content of CPH compared to unfermented CPHs. In conclusion, fermentation of CPH using *A. niger* with an addition of 1.5% of turmeric before the fermentation is the most effective treatment in decreasing the fiber and antinutritional Components In CPH.

Keywords: Antinutritional, Aspergillus niger, Cocoa Pods Husk, Fiber Fraction, Turmeric

# **INTRODUCTION**

Production of fresh cocoa pods in Indonesia in 2020 was 767 thousand tonnes (Syafira, 2022). High cocoa pod production is in line with waste production. Fresh cocoa pods produce the main products in the form of seeds, fruit pods, and placenta with a ratio of 21.74:75.67:2.59% (Wasmun et al. 2016). Cocoa pod husk (CPH) still contains sufficient nutrients that can be used as feed for livestock, which is cheap and available continuously. The chemical composition of CPH is relatively comparable to forage (mainly grass), so CPH

biomass has the potential as an alternative feed to replace forage (Suryanto et al. 2014). Some of the nutritional components of CPH are crude protein (6.3%), crude fiber (24%), fat (0.5%), non-nitrogen extract (32.1%) (Yahya et al. (2015); Adamafio, 2013; Haruna and Rasbawati (2020).

However, CPH has some disadvantages, such as high fiber fraction content, including 68.70% acid detergent fiber (ADF), 75.36% neutral detergent fiber (NDF), 30.25% cellulose, and 6.66% hemicellulose (Agus and Budisatria 2012), which limited its digestibility. These antinutritional compounds in the form of lignin, tannins, and theobromine affect the availability of nutrients. Theobromine is a heterocyclic compound that can inhibit digestion (Adamafio 2013). Efforts to overcome these deficiencies are generally carried out through processing either mechanically, chemically, or biologically to break the bonds between lignocellulose and hemicellulose with lignin and reduce theobromine and tannin content.

The fermentation process is one method that can be used to reduce the antinutrient contents in CPH. Fermentation with A. niger can increase the nutritional content of CPH. A. niger is one of the fast-growing and produces several enzymes such as amylase, pectinase, amyloglucosidase, and cellulase. A. niger produces tannase enzymes that can degrade tannins into soluble gallic acid and glucose (Chávez-González, Rodríguez-Duran, et al. 2018). The fermentation of CPH using A. niger and A. oryzae can reduce the fiber fraction (crude fiber, NDF, and ADF) and increase protein content (Rakhmani and Purwadaria 2018). The lignin content in CPH can also be reduced by using the laccase enzyme oxidizing phenol to phenoxyl. In addition, the laccase enzyme is also able to degrade theobromine which is helpful as a carbon source, nitrogen source, and energy source for A. niger (Zhang et al. 2014).

In the fermentation process, adding herbal plants can also improve the quality of agricultural waste. Using herbal plants, especially turmeric, could improve the quality of feed ingredients from agricultural and plantation waste. According to a study by Khanifah et al. (2021) that combined commercial cocoa products and turmeric in a ratio of 50%:50% showed the presence of flavonoids and tannins, but it did not contain alkaloids. Turmeric rhizome fermented with Aspergillus oryzae significantly increases the antioxidant content compared to those without fermentation (Sulasiyah et al. 2018). Fermentation of turmeric rhizome with EM-4 produced higher levels of curcumin than unfermented turmeric extract, which was analyzed using the TLC-densitometry method (Yasa et al. 2015). These results align with research done by Lim et al. (2021), which showed that fermented turmeric with Rhizopus oligosporus for seven days can increase curcumin phenolics' and antioxidant content. Turmeric contains amylase, lipase, and protease enzymes, which help improve the digestion of feed ingredients such as carbohydrates, fats, and proteins. In addition, turmeric also contains secondary metabolites of flavonoids, alkaloids, and tannins (Sidiq and Wardani, 2014). Based on the description above, it is necessary to study a new approach to reducing the fiber fraction and antinutritional content of CPH by combining *A.niger* and turmeric powder in the fermentation of CPH. The purpose of this study was to evaluate the effectiveness of the fermentation process using *A.niger* with the addition of turmeric powder at different levels in reducing fiber fraction and antinutritional contents of CPH.

### MATERIALS AND METHODS

#### **Materials preparation**

This study was conducted in the Animal Feed Chemistry Laboratory, Department of Animal Nutrition and Animal Feed, Faculty of Animal Science, Hasanuddin University, Makassar, and the Integrated Research and Testing Laboratory at Gajah Mada University from February through April 2022. The materials used in the study were fresh CPH obtained from South Sulawesi Province, Indonesia. The CPH was collected from local types of cocoa trees that were superior in terms of fruit production, fruit quality, and health. The CPH was then immersed in a 0.5% sodium metabisulfite solution for 15 minutes to maintain the phenolic and flavonoid levels in the CPH (Sartini et al. 2017). Furthermore, the CPH was melted down with a grinding process to increase the surface area, and then the CPH was dried under sunshine until dry (moisture content  $\pm 15\%$ ). Turmeric powder and fermenter in the form of A. niger were commercial products that were available in the market. Apart from these three ingredients, water, granulated sugar, urea, and NPK were also used during the incubation period. The equipment to activate the fermenter were buckets with a minimum capacity of 10 liters, aerators, and wooden sticks as stirrers. The activation process of A. niger followed the method of (Fransistika et al. 2013).

#### **Experimental design**

The study was carried out according to a completely randomized design (CRD), which consisted of eight treatments and three replications for each treatment, giving a total number of experimental units of 24. The treatments were P0: CPH without fermentation (Control), P1: Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. Niger*, P2: Fermented (CPH + 0.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. Nige*, P3: Fermented (CPH + 1.0% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. Nige*, P4: Fermented (CPH + 1.5% Turmeric powder) 5 x 10<sup>-3</sup> mL/L *A. Niger*, P5: Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. Niger*, P5: Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 0.5% Turmeric powder, P6: Fermented CPH with5 x 10<sup>-3</sup> mL/L *A. niger* + 1.0% Turmeric powder, P7: Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder.

# Procedures

The CPH fermentation process for P2 to P4 treatments was carried out by spreading CPH and adding turmeric powder according to the treatment with a thickness of 2 cm covering an area of 100 cm<sup>2</sup> and spraying with 5 x 10<sup>-3</sup> mL/L A. niger activation solution until the water content was 40%, then mixed until homogeneous. The CPH fermentation process for treatments P1, P5 to P7 was carried out by spreading CPH with a thickness of 2 cm covering an area of 100 cm<sup>2</sup> and spraying A. *niger* activation solution until the water content was 40%, then mixed until homogeneous. After completion, labeling was conducted to make it easier to control. The fermentation process lasted for seven days with the anaerobic principle. After seven days, the storage area for CPH and turmeric was opened and dried under the sun for 1-2 days to stop the fermentation process. After the next, treatments P5 to P7 were added with turmeric powder according to the treatment. Furthermore, the sieving process was carried out to make a uniform size and put it into a temporary container before analyzing the content of fiber fractions and antinutrients.

#### **Parameters measured**

The variables observed were fiber fraction and antinutrients. The fiber fractions, including NDF, ADF, cellulose, hemicellulose, and lignin, were analyzed using the procedure of Van Soest (1994). The tannin content was determined according to the redox titration analysis method using KMnO<sub>4</sub> reagent Aliwarga & Victoria (2019) in the Animal Feed Chemistry Laboratory, Faculty of Animal Science, Hasanuddin University. The theobromine variable was determined using the HPLC method at the Integrated Research and Testing Laboratory at Gadjah Mada University.

# Data analysis

The data obtained were analyzed using analysis of variance (ANOVA) based on a completely randomized design (Noto 2015). The significant differences among the treatments were further analyzed using contrast orthogonal. All data were analyzed using the statistical package of SPSS version 22.

# **RESULTS AND DISCUSSION**

# Fiber fraction analysis of fermented CPH and turmeric powder by *A. niger*

The data in Table 1 shows that the treatment of CPH fermentation with *A. niger* resulted in significantly lower ADF, NDF, and cellulose values compared to CPH

without fermentation. The reduced content of fiber components due to fermentation using A. niger on CPH was due to the degradation of complex cell walls into simpler components, as well as the dissolution of some proteins and hemicelluloses in the cell walls in organic acid solutions. An organic acid solution can be produced from the fermentation process; this follows Setiawan's (2014) and Peng et al. (2012) opinion that acid solutions hydrolyze hemicellulose. The cellulose-breaking enzymes produced by A. niger, such as the enzymes protease, glucoamylase, lipase, cellulase, amylase, hemicellulase, pectinase, oxidase, and catalase (Yusriani and Puastuti 2020) will make it easier to break down the fiber contained in the fermented substrate, and this follows the opinion of Maulana et al. (2021) that the cellulase enzymes produced by microbes can overhaul the structure of cellulose into more straightforward sugar products that will be useful as an energy source. This phenomenon is followed by the research of Rahmat (2020), which stated that cellulase enzymes produced by microbes can break down the structure of cellulose into more straightforward sugar products, which will be useful as an energy source.

The average results of this study Fermentation treatment compared to fermentation treatment of A. niger added with turmeric powder significantly reduced the content of ADF and hemicellulose compared to CPH, which was fermented without the addition of turmeric powder. The reduced content of ADF and hemicellulose is an indication of the effectiveness of adding turmeric powder to reduce the fiber component of CPH fermented with A. niger. The main nutritional content, flavonoids, and various enzymes in turmeric can streamline the fermentation process so that it has an impact on reducing the fiber component of CPH, which is fermented and added to turmeric powder, that is in line with Berlian et al. (2017); Ergina, Nuryanti, and Pursitasari (2014); Cobra, Amini, and Putri (2019); and Udayani, Ratnasari, and Nida (2022) who explained that the main ingredients in turmeric rhizome include essential oil, curcumin, resin, oleoresin, desmetoxicurcumin, bidesmetoxiccurcumin, fat, protein, calcium, phosphorus, iron, alkaloid, flavonoid and tannin. These compounds are included in the content of secondary metabolites, which can be utilized as antioxidants, anti-inflammatory, anti-blood coagulants, anti-cancer, anti-biotics, and can inhibit carcinogenic effects (Ergina et al. 2014).

The low NDF content in fermented CPH is caused by the breakdown of crude fiber content by *A. niger* mold into organic acids during the fermentation process. As a result, the breakdown of cellulose and the weakening of the crude fiber complex bonds decrease the crude fiber content. Research by (Nurdin et al. 2021) showed that the NDF content decreases due to the production of the cellulase enzyme from *A. niger* during the incubation process. Therefore, the amount of *A. niger* added will

Transforment	Variable			
Treatment	ADF	NDF	Cellulose	Hemicellulose
Control (P0)	65.41	70.13	26.16	4.73
Fermented CPH (P1)	61.87	68.80	25.00	6.93
Fermented (CPH+Turmeric)				
0,5% (P2)	64.03	68.94	25.95	4.91
1,0% (P3)	63.95	66.9	25.65	2.97
1,5% (P4)	62.87	66.65	24.38	4.08
Fermented CPH+Turmeric				
0,5% (P5)	63.57	68.82	25.71	5.24
1,0% (P6)	62.89	71.13	25.34	8.24
1,5% (P7)	62.59	68.21	24.74	5.61
Contrast Test				
P0 Vs. P1, P2, P3, P4, P5, P6, P7	65.42 Vs. 63.11*	70.13 Vs. 68.54*	26.16 Vs. 25.25*	4.73 Vs. 5.42*
P1 Vs. P2, P3, P4, P5, P6, P7	61.88 Vs. 63.32*	68.80 Vs. 68.50 <sup>Ns</sup>	$25.00 \text{ Vs.} 25.29^{\text{Ns}}$	6.93 Vs. 5.17*
P2, P3, P4, Vs. P5, P6, P7	63.62 Vs. 63.02 <sup>Ns</sup>	67.61 Vs.69.39*	$25.33 \ Vs.25.27^{Ns}$	3.99 Vs. 6.37*
P2 Vs. P3	64.03 Vs. 63.95 <sup>Ns</sup>	68.94 Vs. 66.90*	25.95 Vs. 25.65 <sup>Ns</sup>	4.91 Vs. 2.97*
P2 Vs. P4	64.03 Vs. 62.87 <sup>Ns</sup>	68.94 Vs. 66.65*	25.95 Vs. 24.38*	$4.91 \text{ Vs. } 4.08^{N_S}$
P3 Vs. P4	63.95 Vs. 62.87 <sup>Ns</sup>	66.90 Vs. 66.65 <sup>Ns</sup>	25.65 Vs. 24.38*	2.97 Vs. 4.08*
P5 Vs. P6	63.57 Vs. 62.89 <sup>Ns</sup>	68.82 Vs. 71.13*	25.71 Vs. 25.34 <sup>Ns</sup>	5.24 Vs. 8.24*
P5 Vs. P7	63.57 Vs. 62.89 <sup>Ns</sup>	68.82 Vs. 68.21 <sup>Ns</sup>	25.71 Vs. 24.74 <sup>Ns</sup>	5.24 Vs. 5.61 <sup>Ns</sup>
P6 Vs. P7	62.89 Vs. 62.89 <sup>Ns</sup>	71.13 Vs. 68.21*	25.34 Vs. 24.74 <sup>Ns</sup>	8.24 Vs. 5.61*

Table 1. The mean of fiber fraction of fermented CPH and turmeric powder by A. niger

\*= Significantly different (P<0.05), Ns= No significant (P>0.05), ADF= Acid detergent fiber, NDF= Neutral detergent fiber, P0= CPH without fermentation (Control), P1= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P2= Fermented (CPH + 0.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P3= Fermented (CPH + 1.0% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger* + 0.5% Turmeric powder, P6= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder

affect the production of cellulase enzymes, which play a role in breaking cell wall bonds, so that the crude fiber contained in the material will be degraded. *A. niger* mold can produce cellulase enzymes which play a role in breaking down the crude fiber content. The fermentation process also breaks lignin bonds to reduce the NDF content (Ananda 2021).

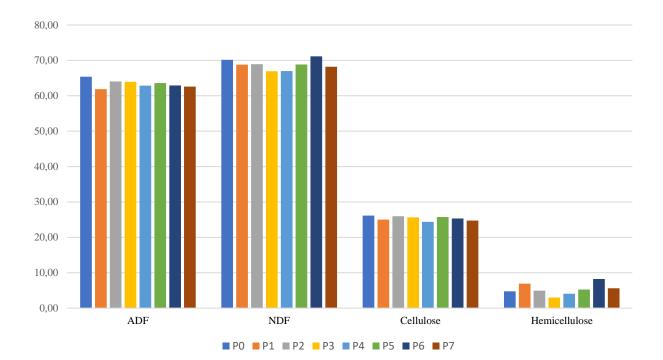
The addition of turmeric to the CPH fermentation process with *A. niger* markedly reduced the fiber components in the form of NDF and hemicellulose compared to turmeric, which was added to CPH without being involved in the fermentation process. These results reinforce the statement that turmeric streamlines the CPH fermentation process. That is also supported by the data shown in Figure 1.

Figure 1 shows that increasing turmeric powder to the CPH fermentation process with increasing levels

decreases the CPH fiber components, especially the NDF, cellulose, and hemicellulose components. This decrease was achieved by using up to 1.5% of turmeric powder, compared to CPH, which was fermented and then added to turmeric. However, the turmeric did not participate in the fermentation process.

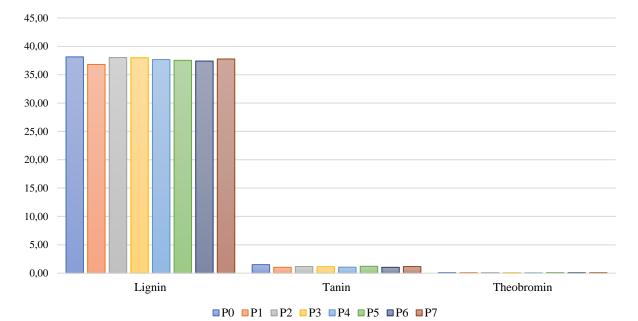
# Antinutritional content of fermented CPH and turmeric using *A. niger*

The data in Table 2 shows that the treatment of CPH fermentation with *A. niger* resulted in significantly lower tannin content and tended to reduce the lignin content compared to CPH without fermentation. The reduced content of antinutritional components due to fermentation using A. *niger* on CPH is due to the



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Figure 1. Average Results of Van Soest of Fermented CPH + Turmeric (%). Means of fiber fractions of CPH according to the treatment. ADF= Acid detergent fiber, NDF= Neutral detergent fiber, P0= CPH without fermentation (Control), P1= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P2= Fermented (CPH + 0.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P3= Fermented (CPH + 1.0% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P5= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P5= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P5= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L A. niger, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L A. niger + 1.5% Turmeric powder



**Figure 2.** The mean of antinutritional components of fermented CPH and Turmeric by *A. Niger*. P0= CPH without fermentation (Control), P1= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P2= Fermented (CPH + 0.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P3= Fermented (CPH + 1.0% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger*, P4= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P6= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10<sup>-3</sup> mL/L *A. niger* + 1.5% Turmeric powder

Transforment	Variable			
Treatment	Lignin	Tannin	Theobromine	
Control (P0)	38.14	1.50	0.11	
Fermented CPH (P1)	37.66	1.05	0.11	
Fermented (CPH+Turmeric)				
0,5% (P2)	38.02	1.16	0.11	
1,0% (P3)	37.98	1.14	0.09	
1,5% (P4)	36.80	1.03	0.07	
Fermented CPH+Turmeric				
0,5% (P5)	37.52	1.20	0.11	
1,0% (P6)	37.40	1.03	0.10	
1,5% (P7)	37.76	1.16	0.10	
Contrast Test				
P0 VS P1, P2, P3, P4, P5, P6, P7	38.14 Vs. 37.59 <sup>Ns</sup>	1.51 Vs.1.11*	0.11 Vs. 0.09 <sup>Ns</sup>	
P1 VS P2, P3, P4, P5, P6, P7	36.80 Vs. 37.72 <sup>Ns</sup>	1.03 Vs. 1.12 <sup>Ns</sup>	0.11 Vs. 0.09 <sup>Ns</sup>	
P2, P3, P4 VS P5, P6, P7	37.89 Vs. 37.56 <sup>Ns</sup>	1.12 Vs. 1.13 <sup>Ns</sup>	0.09 Vs. 0.10 <sup>Ns</sup>	
P2 Vs. P3	38.02 Vs. 37.98 <sup>Ns</sup>	1.16 Vs. 1.14 <sup>Ns</sup>	0.11 Vs. 0.09 <sup>Ns</sup>	
P2 Vs. P4	38.02 Vs. 36.80*	1.16 Vs. 1.03 <sup>Ns</sup>	0.11 Vs. 0.07*	
P3 Vs. P4	37.98 Vs. 36.80*	1.14 Vs. 1.03 <sup>Ns</sup>	$0.09 \text{ Vs. } 0.07^{\text{Ns}}$	
P5 Vs. P6	37.52 Vs. 37.40 <sup>Ns</sup>	1.20 Vs. 1.03*	0.11 Vs. 0.10 <sup>Ns</sup>	
P5 Vs. P7	37.52 Vs. 37.76 <sup>Ns</sup>	1.20Vs. 1.16 <sup>Ns</sup>	0.11 Vs. 0.10 <sup>Ns</sup>	
P6 Vs. P7	37.40 Vs. 37.76 <sup>Ns</sup>	1.03 Vs. 1.16 <sup>Ns</sup>	0.10 Vs. 0.10 <sup>Ns</sup>	

Table 2. Analysis results of antinutritional content of fermented CPH and turmeric using A. niger

\*= Significantly different (P<0.05), Ns= No significant (P>0.05), P0= CPH without fermentation (Control), P1= Fermented CPH with 5 x  $10^{-3}$  mL/L *A. niger*, P2= Fermented (CPH + 0.5% Turmeric powder) with 5 x  $10^{-3}$  mL/L *A. niger*, P3= Fermented (CPH + 1.0% Turmeric powder) with 5 x  $10^{-3}$  mL/L *A. niger*, P5= Fermented CPH with 5 x  $10^{-3}$  mL/L *A. niger*,

degradation of complex antinutrients into simpler components, as well as the hydrolysis of tannins by the tannase enzyme produced from the fermentation process. Tannase enzymes can be produced from a solid phase fermentation process with A. niger 1.401 U/mL (Chávez-González, Guyot, et al. 2018). Tannase is an extracellular enzyme produced by several microorganisms, such as bacteria, yeast, and fungi. Research by Lekshmi et al. (2021) suggests that the fungus genera Aspergillus and Penicillium are the best producers of the tanase enzyme. Rakhmani and Purwadaria (2018) fermentation with A. niger produces more mannanase and cellulase enzymes than Aspergillus oryzae. These enzymes help break down and loosen lignocellulosic bonds; high cellulose can reduce the lignin content. The trend of decreasing lignin content was due to the breakdown of lignocellulose bonds by cellulase enzymes produced by A. niger during the fermentation process. Lignocellulosic and hemicellulose bonds in the cell walls of cocoa pod shells will be stretched in the presence of lignocellulose and cellulase enzymes produced by A. niger, that aligns with the statement (Dalimunthe et al. 2021), which states that fermentation aims to break down lignocellulosic complex bonds and produce cellulose content to be broken down by cellulase enzymes produced by microbes. Fiber treatment in akaa leaves heated in turmeric solution can increase cellulose levels and reduce lignin levels, while hemicellulose levels are almost constant (Renreng et al. 2017). Lignin is a phenylpropanoid polymer that is challenging to break down because of its heterogeneous and very complex structure. The composition of plant material is 30% lignin. Therefore, lignin can provide strength to wood against attacks by microorganisms (Carniel et al. 2021).

Fermentation treatment compared to *A. niger* fermentation treatment added with turmeric powder had no significant effect on the antinutritional components of CPH. The absence of anti-nutrition content indicates that the addition of turmeric powder in CPH fermentation with *A. niger* has not been effective in reducing tannins, lignin, and theobromine in CPH. The addition of turmeric to the CPH fermentation process with *A. niger* and turmeric added to CPH without being involved in the fermentation process did not significantly affect the decrease in the antinutritional content of CPH.

Figure 2 shows that increasing turmeric powder to the CPH fermentation process impacts reducing the antinutritional components of CPH in the tannin 11.20%, lignin 2.54%, and theobromine 29.00% components. This decrease was achieved by using up to 1.5% of turmeric powder, compared to CPH, which was fermented and then added to turmeric. However, the turmeric did not participate in the fermentation process. The trend of decreasing theobromine content at treatment levels using turmeric powder up to 1.5%, which A. niger ferments, indicates that theobromine degradation by A. niger occurs via the demethylase route and involves the expression of enzymes such as theobromine theobromine demethylase, oxidase, xanthine dehydrogenase, xanthine oxidase, urease and uricase Yamaoka-Yano and Mazzafera (1999); Summers et al. (2015); and Cornelis et al. (2016). Some of these statements indicate that A. niger microbes can degrade theobromine (Bentil et al. 2015).

# CONCLUSION

Compared to unfermented CPH, the addition of turmeric powder up to 1.5% to the CPH fermentation using *A. niger* significantly impacts decreasing the CPH fiber components, especially the NDF, ADF, and cellulose. The impact is more profound in reducing NDF fraction when the turmeric is added to CPH before the fermentation process than adding turmeric after the fermentation of CPH using *A. niger*. Concerning the antinutrient components, the addition of turmeric powder up to 1.5% to the CPH fermentation using *A. niger* only has a profound effect on reducing the tannin content of CPH and tends to decrease the lignin component. Adding turmeric either before or after the fermentation of CPH using *A. niger* has similar effects.

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