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CHARACTERIZATION OF FERMENTED PALM KERNEL CAKE USING LOCALLY ISOLATED CELLULOLYTIC FUNGI AND BACTERIA AS POTENTIAL ANIMAL FEED

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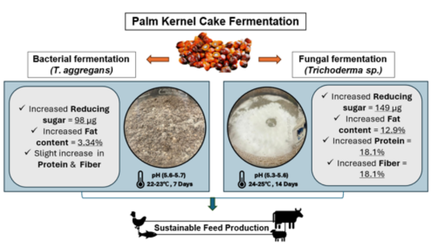
Graphical abstract



Abstract

This study investigates the potential of locally isolated cellulolytic fungi and bacteria for fermenting palm kernel cake (PKC) as animal feed. The characterization includes physical observations, proximate analysis, reducing sugar, hemicellulose, and cellulose content, and cellulase activity. Bacterial fermentation was conducted for 7 days, while fungal fermentation extended to 14 days. The findings reveal comparable pH stability of fermented PKC of both bacteria and fungi (bacteria: 5.6-5.7, fungi: 5.3-5.6). Fungal fermentation resulted in a significant increase in crude fat (9.16% to 12.9%), protein (16.3% to 18.1%), and fiber content (14.3% to 18.1%) of the PKC. Conversely, bacterial fermentation led to a notable decrease in fat content (9.16% to 3.34%), a slight increase in protein, and fiber content of the PKC. Significantly higher reducing sugar levels were observed in fungal saccharification (149 µg) compared to bacterial (98 µg) on day 7 of fermentation. Efficient saccharification, as indicated by the cellulase activity in both bacterial and fungal fermentations, resulted in a decrease in hemicellulose and cellulose content over time. The findings suggest that fungal or bacterial fermentation of PKC can be utilized to customize feed formulations to meet the specific nutritional requirements of animals. This study provides valuable insights into optimizing fermentation conditions to enhance the nutritional value and digestibility of PKC for sustainable feed production.

Keywords: Palm kernel cake, fungi, bacteria, cellulolytic, animal feed

Abstrak

Kajian ini menyelidik potensi kulat dan bakteria selulolitik yang diasingkan secara tempatan untuk menapai kek biji sawit (PKC) sebagai makanan haiwan. Penciran meliputi pemerhatian fizikal, analisis proksimat, gula penurun, kandungan hemiselulosa dan selulosa, serta aktiviti selulase. Penapaian bakteria dijalankan selama 7 hari, manakala penapaian kulat dilanjutkan sehingga 14 hari. Hasil kajian menunjukkan kestabilan pH yang hampir sama bagi PKC yang diperam oleh kedua-dua bakteria dan kulat (bakteria: 5.6-5.7, kulat: 5.3-5.6). Penapaian kulat mengakibatkan peningkatan ketara dalam lemak kasar (9.16% kepada 12.9%), protein (16.3% kepada 18.1%), dan kandungan serat (14.3% kepada 18.1%) dalam PKC. Sebaliknya, penapaian bakteria menyebabkan penurunan ketara dalam kandungan lemak (9.16% kepada 3.34%), serta sedikit peningkatan dalam protein dan serat PKC. Tahap gula penurun yang lebih tinggi secara signifikan diperolehi menggunakan sakarifikasi kulat (149 µg) berbanding bakteria (98 µg) pada hari ke-7 penapaian. Sakarifikasi yang berkesan, seperti yang ditunjukkan oleh aktiviti selulase dalam kedua-dua penapaian bakteria dan kulat, menyebabkan penurunan dalam kandungan hemiselulosa dan selulosa dari masa ke masa. Penemuan ini mencadangkan bahawa penapaian PKC dengan kulat atau bakteria boleh digunakan untuk menyesuaikan formulasi makanan bagi memenuhi keperluan pemakanan spesifik haiwan. Kajian ini memberikan data penting tentang pengoptimuman keadaan penapaian untuk meningkatkan nilai pemakanan dan kebolehcernakan PKC bagi pengeluaran makanan yang mampan.

Kata kunci: Kek biji sawit, kulat, bakteria, selulolitik, makanan haiwan

1.0 INTRODUCTION

There is a continuous rise in food demand driven by the surge in a growing population. The United Nations reported the world's population is projected to grow by 2 billion over the next three decades, reaching 9.7 billion by 2050 [1]. According to Fróna *et al.*, [2] food production in agricultural industries is expected to rise by approximately 60% in the next 40 years to meet the demands of the growing population. Consequently, farmers are facing heightened demand for animal ingredients to support livestock, poultry, and fish nutrition industries.

Livestock serves as a primary protein source meeting major of the total protein demand in human diet [3]. Ensuring an abundant supply of feed is highly crucial in order to ensure a thriving livestock industry. Nevertheless, it faces challenges due to the rising cost of feed which constitutes 70% of the production expenses [4]. Additionally, environmental concerns such as ammonia leaching from chemical-based feeds, greenhouse gas emissions, deforestation, and water pollution emphasize the urgent necessity to explore sustainable and eco-friendly feeds [5].

Palm kernel cake (PKC) is a valuable by-product derived from the palm oil industry. PKC possesses notable protein and fiber content making it beneficial for livestock and animal nutrition [6]. However, its utilization as animal feed is influenced by factors such as the presence of anti-nutritional factors and elevated levels of crude fat, which may limit its suitability in certain animal diets, as reported by Alagawany *et al.*, [7]. High crude fat content can disrupt the diet balance of feeds and reduce nutrient absorption, while anti-nutritional factors such as phytates and tannins hinder mineral absorption and protein utilization by the animals. PKC also contains high cellulose and hemicellulose content [8] that need to be broken down into smaller components for efficient nutrient utilization. Hence, further processing method could increase its potential as feed ingredient.

Cellulolytic microorganisms like bacteria and fungi are promising to overcome these limitations through solid-state fermentation (SSF). Through the actions of specific microorganisms, they can break down complex polysaccharides in PKC cell walls into simpler carbohydrates and sugars via saccharification process, thereby enhancing the availability and digestibility of nutrients in cellulosic materials for animal consumption [9]. Proximate analysis reveals increased crude protein levels and reduced crude fat content [10]. *Trichoderma reesei* and *Aspergillus oryzae* in the study Sun *et al.*, [11] improved the feeding value of wet corn distillers' grains and solubles and soybean hull as feed ingredients for monogastric animals. Lee *et al.*, [12] have isolated lactic acid strains from fermented food to facilitate the biotransformation of PKC. The study demonstrated remarkable activities of extracellular proteolytic, β-glucosidase, β-mannosidase, and β-mannanase enzymes, which contributed to the breakdown of cellulosic biomass. Reducing sugar and cellulase activity levels following fermentation suggests efficient breakdown of nutrients during the fermentation process. According to Zeng *et al.*, [13], elevated reducing sugar levels commonly indicate heightened enzymatic cellulase activity that is responsible for converting cellulose and hemicellulose into simpler sugars. As reducing sugar levels increase due to cellulase activity, the substrate's cellulose and hemicellulose content typically diminishes. Therefore, incorporating SSF in the processing of PKC can significantly increase its value and functionality as a feed resource for livestock.

The present study aimed to evaluate the potential of our previously reported locally isolated cellulolytic *Trichoderma* sp fungi and *Thermoflavilum aggregans* bacteria [14] in maximizing the nutritive value of PKC as a potential animal feed. PKC fermentation using bacteria and fungi was conducted for 7 days and 14 days, respectively. The effects of bacterial and fungal fermentation on proximate analysis, cellulose and hemicellulose content, reducing sugar, and cellulase activity of PKC biomass were investigated. Additionally, changes in PKC morphology were also observed. The findings of this study provide valuable insights into improving the nutritional value of PKC through fermentation techniques. This could facilitate the development of environmentally friendly and economically viable feed production practices in the agricultural industry.

2.0 METHODOLOGY

2.1 Strain and Materials Preparation

The solid-state fermentation of palm kernel cake (PKC) was conducted using locally isolated cellulolytic *T. aggregans* bacteria and *Trichoderma* sp fungi. *T. aggregans* bacteria were cultured in nutrient agar and incubated at 30 °C. *Trichoderma* sp was cultured on potato dextrose agar and incubated at 30 °C. PKC was supplied from Kolej Vokasional Lahad Datu, Sabah. All Apparatus, culture medium, and PKC were autoclaved for sterilization before the solid-state fermentation [14].

2.2 Bacterial and Fungal Fermentation of PKC

The solid-state fermentation process was carried out following the methodology described by Arsy *et al.,* [15] with some modifications. Approximately 20 g of sterilized PKC was placed in an Erlenmeyer flask and inoculated with 4 ml of bacterial culture for bacterial fermentation. The mixture was homogenously mixed using a sterilized spatula and incubated at 30 ℃ for 7 days. Samples were collected on day 0, day 3, and day 7 for morphological and biochemical analyses. For fungal fermentation, approximately 1 cm × 1 cm pieces of fungi grown on PDA media were cut and added to 20 g of sterilized PKC in an Erlenmeyer flask, then incubated at 30 ℃ for 14 days without aeration. Fermentation was carried out using the original moisture content of the PKC. The experiments were conducted at original moisture conditions of PKC. Samples were collected on day 0, day 7, and day 14 for morphological and biochemical analyses. All samples were conducted in triplicates.

2.3 Morphological and Chemical Analysis

2.3.1 Morphological Observation

The morphological observation of bacterial-fermented and fungal-fermented PKC were observed on day 7. The pH of the PKC sample was measured using pH meter while the temperature was monitored by using thermometer.

2.3.2 Determination of Proximate Analysis

Content of crude protein, moisture, ash and crude fat of the samples measured under standard methods described by the Horwitz and Latimer [16].

2.3.3 Determination of Reducing Sugar

An amount of reducing sugars was determined according to Alshelmani *et al.,* [17], whereby the fermented samples in conical flask was added with 20 mL sterile distilled water followed by agitation for an overnight in a rotary shaker at 130 rpm in 25°C. The solution was then filtrated using Whatman filter paper number 1 and then centrifuged at 10,000 g, 4°C for 15 min. The supernatant was kept at −20°C for further analysis. The sugar standard was then determined using dinitro salicylic acid reagent according to Miller [18].

2.3.3 Determination of Hemicellulose and Cellulose Content

The hemicellulose and cellulose content were assessed following the method outlined by Alshelmani *et al.,* [17], which involved measuring the total acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL). All three components (ADF, NDF, and ADL) were quantified using the procedure established by Goering and Van Soest [19]. Cellulose content was determined by subtracting the ADL value from ADF, while hemicellulose content was calculated by subtracting ADF from NDF.

2.3.3 Determination of Cellulase Activity

Cellulase activity was determined according to Alshelmani *et al.,* [17]. In brief, a modified dinitro salicylic acid (DNS) reagent lacking phenol and sodium sulfite was utilized, and potassium sodium tartrate was not introduced separately. Substrates were dissolved in buffers at ambient temperature. The crude enzyme was incubated with substrate for 60 minutes, followed by the addition of 2 mL of DNS reagent to halt the reaction. Test tubes were then boiled for 5 minutes and subsequently cooled in running ice water for another 5 minutes. Standard reference for glucose was employed, and absorbance was measured at 540 nm using a spectrophotometer. Enzyme activity was calculated based on reducing sugars, with specific enzyme activity defined as the quantity of enzyme capable of liberating reducing sugar in μmol per minute per mg of protein under assay conditions.

2.4 Statistical Analysis

All measurements were performed in triplicate. Statistical analysis was performed using a one-way ANOVA to evaluate the effects of the fermentation type (Bacterial vs. Fungal) on increasing the nutritional value of PKC and was further assessed through a *t*-test. All statistical tests were conducted at a significance level of α = 0.05. Differences were considered statistically significant at $p < 0.05$. The analysis was performed using Microsoft Excel Software.

3.0 RESULTS AND DISCUSSION

3.1 Physical Observation

PKC was subjected to bacterial fermentation for 7 days, while fungal fermentation was for 14 days, as fungi are known to have slower growth rates compared to bacteria [20]. Observations of bacterial-fermented PKC were made on days 0, 3, and 7, while fungal fermentation observations were conducted on days 0, 7, and 14. Figure 1 illustrates the morphological changes between non-fermented PKC (Figure 1a) and fermented PKC (Figure 2b-c). Bacterial fermentation exhibited subtle alterations resulting in a lighter PKC appearance (Figure 1b), whereas fungal fermentation induced more pronounced changes. The PKC displayed alterations in the texture and color, with the particles becoming softer and developing a gel-like texture, as shown in Figure (c).

The changes in pH and temperature of PKC biomass were monitored throughout the fermentation process. As depicted in Figure 2(a), the temperature of PKC during bacterial fermentation consistently remained in the range of 22°C to 23°C. Fungal fermentation exhibited a slightly higher temperature consistently, ranging from 24°C to 25°C over the 14 days, indicating sustained microbial activity throughout the longer fermentation duration. Throughout the 7-day bacterial fermentation and 14-day fungal fermentation, the pH of PKC remained stable at around 5.3 – 5.7. This consistent pH level indicates a balanced and favorable environment for the fermenting microorganisms, supporting their growth and efficient enzymatic processes during both fermentation processes [21]. Further analyses, such as proximate analysis, cellulase activity, and other biochemical assessments, were conducted to evaluate the fermentation process and its effects on PKC.

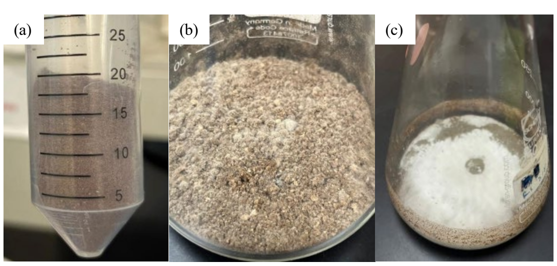


Figure 1 Morphological observation of (a) Non-fermented PKC, (b) bacteria-fermented PKC, (c) fungi-fermented PKC on day 7 of fermentation.

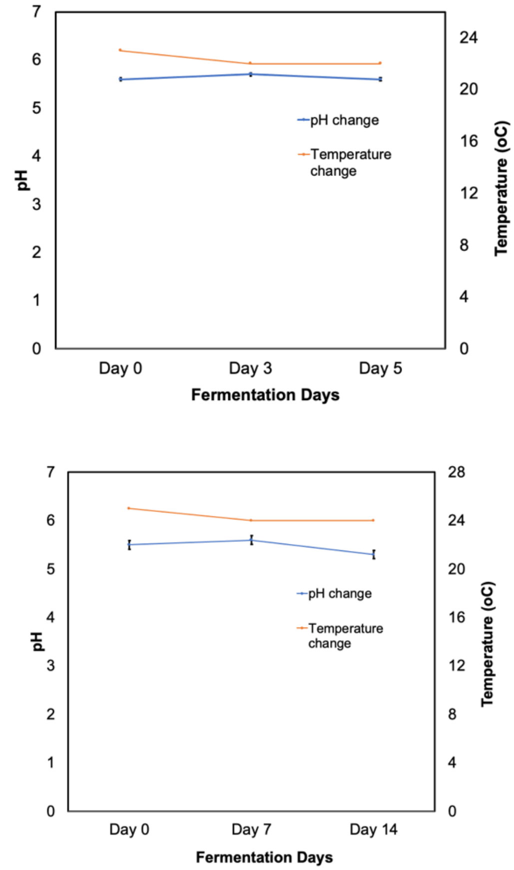


Figure 2 pH and temperature differences of (a) bacterial-fermented PKC and (b) fungal-fermented PKC throughout the fermentation process

3.2 Proximate Analysis of Fermented PKC

The proximate analysis results for bacterial and fungal fermentation of PKC revealed distinctive effects on its nutritional composition (Table 2). Bacterial fermentation showed a decline in crude fat content from 9% to 3.5%, while crude protein content increased gradually from 16.2% to 17.48% by day 7. Ash content remained relatively stable, ranging from 4.46% to 4.87%, with crude fiber showing minor fluctuations from 14.66% to 15.19% before settling at 14.91%. In contrast, fungal fermentation decreased PKC's ash content from 4.78% to 3.93% by day 14 and increased crude fat content from 9.16% to 12.93%. Crude protein and crude fiber displayed slight variations over the 14-day fermentation period, with the crude fiber content from fungal fermentation falling within the acceptable range for most ruminants (16-18%), as reported by Azizi *et al.,* [6].

Table 1 Proximate analysis of fungal-fermented PKC in percentage

Day	Ash	Fat	Protein	Fiber
Fungal-Fermented PKC				
0	4.78 ± 0.27	9.16 ± 0.18	16.28 ± 0.40	14.28 ± 0.43
7	4.51 ± 0.17	10.58 ± 0.31	16.05 ± 0.29	16.60 ± 0.09
14	3.93 ± 0.08	12.93 ± 0.35	18.14 ± 0.33	18.11 ± 0.83
Bacterial-Fermented PKC				
0	4.78 ± 0.27	9.16 ± 0.17	16.2 ± 80.40	14.66 ± 0.43
3	4.46 ± 0.22	3.53 ± 0.38	16.94 ± 0.14	15.19 ± 0.39
7	4.87 ± 0.26	3.34 ± 0.06	17.48 ± 0.53	15.91 ± 0.66

Data represent the average of 3 replicates (n=3).

Overall, both bacterial and fungal fermentations of PKC had distinct effects on its proximate analysis. Bacterial fermentation led to a decrease in crude fat content and an increase in crude protein content, suggesting improved nutritional value as a protein source for animals and addressing the limitation of high-fat content in some diets. Barros *et al.*, [22] have reported that cocoa pod husk fermentation with the fungal strain *Rhizopus stolonifera* showed an increment of soluble protein level from 8.71 mg/g to 11.33 mg/g. While the impact on ash and crude fiber content was relatively minor in this study, fungal fermentation reduced ash content and significantly increased crude fat content, which potentially enhances PKC's suitability as a balanced and desirable animal feed. These findings offer promising opportunities for fermented PKC in animal nutrition. Further optimization of the fermentation process can be pursued to obtain the desired properties of the PKC.

3.3 Hemicellulose and Cellulose Content of Fermented PKC

The reducing sugar content, cellulase activity, hemicellulose content, and cellulose content of the fermented PKC were further analysed. These analyses are vital to demonstrate the breakdown of hemicellulose and cellulose in PKC into simpler sugars and to measure the activity of cellulase enzymes responsible for the breakdown [23]. The results showed changes in hemicellulose and cellulose content of bacterial (Figure 3a) and fungal (Figure 3b) fermentation of PKC. Both bacterial and fungal fermentation resulted in a gradual reduction of hemicellulose over time. Bacterial fermentation led to a decrease of hemicellulose content from 29% to 24% on day 7 (Figure 3a), while fungal fermentation showed a decrease from 29% to 14.3% on day 14 (Figure 3b).

A similar trend was observed for the cellulose content in both bacterial (Figure 3a) and fungal fermentation (Figure 3b). The cellulose content decreased over time, with bacterial fermentation showing a decrease from 37% on day 1 to 26% on day 7, and fungal fermentation exhibiting a decrease from 37% on day 0 to 12% on day 14. The findings suggest that both bacteria and fungi were capable of breaking down hemicellulose and cellulose during fermentation.

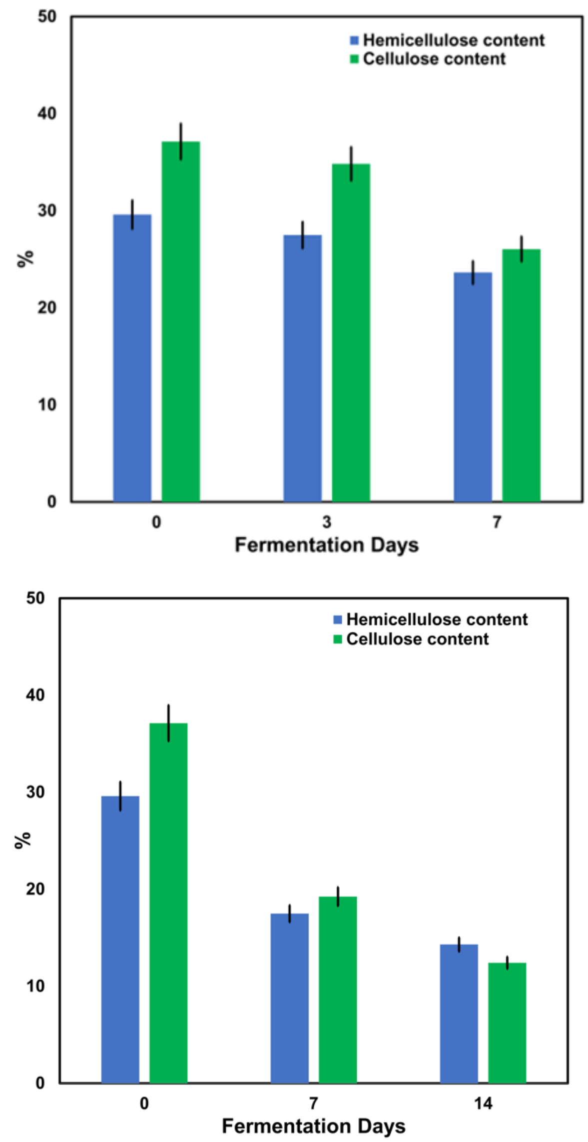


Figure 3 Hemicellulose and cellulose content of (a) bacterial-fermented PKC and (b) fungal-fermented PKC. *Data represent the average of 3 replicates (n=3)*

Azizi *et al.*, [6] reported that diverse cellulolytic bacteria and fungi can catalyze the hydrolysis of lignocellulose within plant cell walls. Fungi demonstrated superiority over bacteria in degrading hemicellulose and cellulose, even at the same duration of fermentation. On day 7, bacterial fermentation led to a 17% reduction in hemicellulose content and a 10% reduction in cellulose content, whereas fungal fermentation showed a more significant degradation, with a 40% reduction in hemicellulose and a 34% reduction in cellulose content. According to Weimer [24], cellulose and hemicellulose are degraded and fermented by anaerobic microbes in the animal rumen to produce volatile fatty acids as the main nutrient source. The study observed that with longer fermentation periods, both bacterial and fungal fermentations resulted in higher reductions of cellulose and hemicellulose.

3.4 Reducing Sugar of Fermented PKC

The reducing sugar content of both bacterial and fungal fermentation are presented in Figure 4. The PKC fermentation with bacteria showed a gradual increase in reducing sugar, starting at 72 µg on day 0, reaching 74 µg on day 3, and further increasing to 98 µg on day 7 (Figure 4a). The rapid increase on day 7 could be attributed to the bacteria being in the exponential growth phase, leading to active metabolic production and higher sugar accumulation [25]. On the other hand, fungal fermentation showed a different trend, with a reducing sugar content increased up to 149 µg on day 7 but reduced to 95 µg on day 14 (Figure 4b). During the initial stages of fermentation, when nutrients are abundant, the fungi rapidly break down complex carbohydrates present in the palm kernel cake, resulting in a higher release of reducing sugars. This is evident by the drastic degradation of cellulose on day 7, from 37% to 19%, which contributed to nearly 50% of the cellulose reduction. The fermentation of PKC by *Trichoderma longibrachiatum* resulted in a substantial decrease in cellulose levels from 28.31% to 12.11% [26]. As the fermentation progressed and reached day 14, the fungi might have depleted some of the readily available nutrients, leading to a decline in their metabolic activity. By day 14 of fungi fermentation, the metabolic activity likely decreased due to the depletion of readily available nutrients. As described by Tse *et al.,* [27], microbial fermentation converts complex organic molecules into simpler ones, releasing fermentable sugars such as monosaccharides and disaccharides through enzymatic hydrolysis. During fermentation, the fermentative microbes enhance reducing sugar availability through the secretion of hydrolytic enzymes such as cellulases, hemicellulases, and xylanases, which break down complex polysaccharides into simple sugars. These sugars are then utilized through microbial metabolic pathways like glycolysis and the TCA cycle, supporting growth and sustained enzyme production [28]. The decrease in reducing sugar content could be a consequence of the fungi utilizing the available sugars for their own growth and energy requirements, as well as other metabolic processes. As fungi proliferate, they consume available reducing sugars as primary carbon and energy sources to support cellular processes, including metabolism and sporulation. This consumption leads to a measurable decrease in reducing sugar concentrations over time [29]. These findings highlight the significant influence of fermentation duration on achieving desired product outcomes.

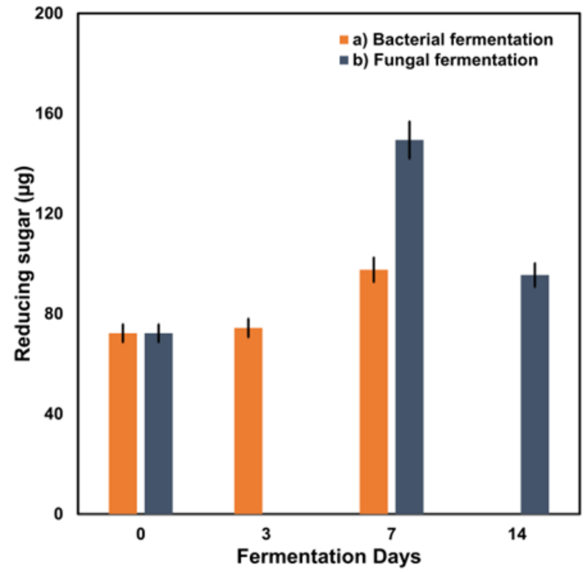


Figure 4 Reducing sugar of (a) bacterial -fermented PKC and (b) fungal-fermented PKC. *Data represent the average of 3 replicates (n=3)*

3.5 Cellulase Activity of Fermented PKC

Cellulase is an essential enzyme required for biotransformation of PKC during solid state fermentation [12]. The cellulase activity of both bacterial and fungal fermentation on PKC was measured in units per gram of PKC (U/g PKC) (Figure 5). On day 3 of bacterial fermentation, the cellulase activity was recorded as 0.8 U/g PKC and significantly increased to 6.8 U/g PKC on day 7 (Figure 5a). This increase in cellulase activity correlates with the trend of reducing sugar production, which was almost negligible on day 3 but showed a significant increase on day 7 (Figure 4a). During fungal fermentation, the cellulase activity was higher, reaching 11 U/g PKC on day 7, but slightly decreased to 7 U/g PKC on day 14 (Figure 5b). This observation aligns with the trend of reducing sugar production, showing a 1.57-fold reduction from day 7 to day 14 (Figure 5b). These findings highlight the significant influence of fermentation duration on achieving desired product outcomes. The higher cellulase activity of fungal fermentation (11 U/g PKC) compared to bacteria (6.8 U/g PKC) on day 7 is consistent with above-mentioned fungal's superiority in degrading hemicellulose and cellulose (Figure 3b). This finding is also supported by a study by Ng and Zeikus [28], comparing extracellular cellulase activities of *Clostridium thermocellum* LQRI and *Trichoderma reesei* QM9414, which found fungal cellulolytic enzymes to be superior to bacterial enzymes in terms of cellulase activity. Fasuyi *et al.,* [29] reported inclusion of cellulase, and other fibrolytic enzymes in broilers' diets enhances the breakdown of the diet and subsequently improves the growth performance of birds.

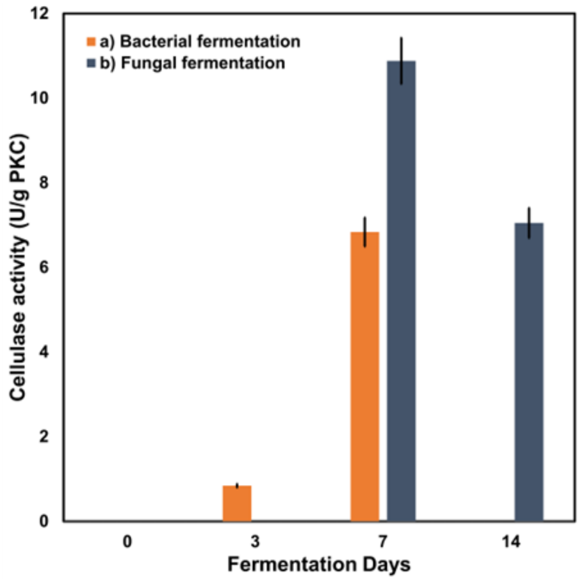


Figure 5 Cellulase activity of (a) bacterial-fermented PKC and (b) fungal-fermented PKC. *Data represent the average of 3 replicates (n=3)*

The results reveal significant biochemical changes during bacterial and fungal fermentations of PKC. Fungi demonstrate superior saccharification capacity and efficient breakdown of hemicellulose and cellulose, suggesting their potential in improving PKC's nutritive value and digestibility as sustainable livestock feed. When compared to previously reported studies in Table 3, this study shows favorable results, indicating increased protein, fat, and crude fiber content in the fermented PKC compared to other agro-wastes fermented with different microorganisms. Moreover, the statistical report shown in Table 4 reveals the nutritional value content between bacterial and fungal fermentation. The report has shown significant differences between crude fat, hemicellulose and cellulose content. However, other nutritional content showed marginal significance, or there was no significant difference between the types of microbial fermentation. To conclude, fungal fermentation is superior compared to bacterial fermentation in enhancing the nutritional values for crude fat and fibre contents (Table 2).

Table 2 Comparison of proximate analysis of various fermented agro-wastes using different types of microorganisms in solid state fermentation

Substrate	Microorganism	Protein	Fat	Crude fiber	Reference
Palm kernel cake	*Trichoderma sp*	18.1±0.33	12.9±0.35	18.1±0.83	This study
Palm kernel cake	*Thermoflavilum aggregans*	17.5±0.53	4.87±0.06	15.9±0.66	This study
Palm kernel cake		16.1	-	15.2	[30]
Palm Kernel Cake	*Rhizopus stolonifer* LAU 07	26.3	7.1	12.5	
Casava peel		19.0	3.7	13.5	
Cocoa pod husk		16	4.2	16.9	
Soybean meal	Aspergillus niger	44.0	1.80	-	[31]
Palm kernel cake	Aspergillus niger	12.1	14.6	-	
Coir pith	Aspergillus niger	1.05	0.26	-	

Table 3 Statistical analysis between bacterial and fungal fermentation on the amount of nutritional value difference.

Nutrient Content	F-value	*p*-value	*t*-test	Interpretation
Crude Fat	25.11	0.0001	5.01	Significant
Crude Protein	0.04	0.853	-0.19	Not Significant
Crude Fiber	3.45	0.078	1.88	Marginal
Reducing Sugar Content	3.98	0.063	1.99	Marginal
Hemicellulose Content	6.24	0.024	-2.50	Significant
Cellulose Content	5.67	0.03	-2.38	Significant

4.0 CONCLUSION

The study investigated the effects of bacterial and fungal fermentations on the nutritional and chemical content of palm kernel cake (PKC) as potential animal feed. Fungal fermentation significantly increased crude fat, protein, and fiber content, while bacterial fermentation 64% reduction in crude fat content and slightly increased crude protein and fiber. Fungal fermentation demonstrated superior efficiency in breaking down hemicellulose and cellulose into reducing sugars and exhibited higher cellulase activity compared to bacterial fermentation, highlighting its potential for improving PKC's digestibility. However, longer fermentation periods beyond 7 days led to reductions in reducing sugar content and cellulose activity. Overall, fungal fermentation shows promise for enhancing PKC's nutritive value and digestibility as a sustainable livestock feed. Nevertheless, the findings suggest that either fungal or bacterial fermentation of PKC can be employed based on specific dietary requirements and animal species to maximize the benefits of PKC as an essential animal nutrition resource. Further optimization of the fermentation conditions, including variables such as temperature, moisture content, and inoculum size, to enhance bioconversion efficiency. Additionally, feeding trials using the fermented product in actual livestock diets are recommended to evaluate its nutritional performance and practical applicability in animal production systems. This study shed light on the potential of fungal and bacterial fermentations to improve the nutritional composition and digestibility of PKC for sustainable livestock feed.

Acknowledgement

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] Yarnold, J., H. Karan, M. Oey, and B. J. Hankamer. 2019. Microalgal Aquafeeds as Part of a Circular Bioeconomy. *Trends in Plant Science*. 24(10): 959–970. https://doi.org/10.1016/j.tplants.2019.06.005.

[2] Fróna, D., J. Szenderák, and M. Harangi-Rákos. 2019. The Challenge of Feeding the World. *Sustainability*. 11(20): 5816. https://doi.org/10.3390/su11205816.

[3] Henchion, M., M. Hayes, A. M. Mullen, M. Fenelon, and B. Tiwari. 2017. Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. *Foods*. 6(7): 53. https://doi.org/10.3390/foods6070053.

[4] González, N., M. Marquès, M. Nadal, and J. L. Domingo. 2020. Meat Consumption: Which Are the Current Global Risks? A Review of Recent (2010–2020) Evidences. *Food Research International*. 137: 109341. https://doi.org/10.1016/j.foodres.2020.109341.

[5] Gržinić, G., A. Piotrowicz-Cieślak, A. Klimkowicz-Pawlas, R. L. Górny, A. Ławniczek-Wałczyk, L. Piechowicz, E. Olkowska, M. Potrykus, M. Tankiewicz, M. Krupka, G. Siebielec, and L. Wolska. 2023. Intensive Poultry Farming: A Review of the Impact on the Environment and Human Health. *Science of the Total Environment*. 858: 160014. https://doi.org/10.1016/j.scitotenv.2022.160014.

[6] Azizi, M. N., T. C. Loh, H. L. Foo, and E. L. Teik Chung. 2021. Is Palm Kernel Cake a Suitable Alternative Feed Ingredient for Poultry? *Animals*. 11(2): 338. https://doi.org/10.3390/ani11020338.

[7] Alagawany, M., S. S. Elnesr, M. R. Farag, R. Tiwari, M. I. Yatoo, K. Karthik, I. Michalak, and K. Dhama. 2020. Nutritional Significance of Amino Acids, Vitamins and Minerals as Nutraceuticals in Poultry Production and Health: A Comprehensive Review. *Veterinary Quarterly*. 41(1): 1–29. https://doi.org/10.1080/01652176.2020.1857887.

[8] Koranteng, A. A. A., K. A. Gbogbo, B. Adjei-Mensah, T. Bouassi, C. T. F. Aïna, J. Glago, and T. Kokou. 2022. Impact of Palm Kernel Cake with or without Multi-Blend Enzyme on the Growth Performance and Carcass Traits of Sasso Broilers. *International Journal of Veterinary Science and Medicine*. 10(1): 80–89. https://doi.org/10.1080/23144599.2022.2125735.

[9] Lu, H., V. Yadav, M. Zhong, M. Bilal, M. J. Taherzadeh, and H. M. N. Iqbal. 2022. Bioengineered Microbial Platforms for Biomass-Derived Biofuel Production: A Review. *Chemosphere*. 288: 132528. https://doi.org/10.1016/j.chemosphere.2021.132528.

[10] Kari, Z. A., S. A. M. Sukri, N. D. Rusli, K. Mat, M. B. Mahmud, N. N. A. Zakaria, W. Wee, N. K. A. Hamid, M. A. Kabir, N. S. N. A. Ariff, S. Z. Abidin, M. K. Zakaria, K. W. Goh, M. I. Khoo, H. V. Doan, A. Tahiluddin, and L. S. Wei. 2023. Recent Advances, Challenges, Opportunities, Product Development and Sustainability of Main Agricultural Wastes for the Aquaculture Feed Industry: A Review. *Annals of Animal Science*. 23(1): 25–38. https://doi.org/10.2478/aoas-2022-0082.

[11] Sun, X., N. Debeni Devi, P. E. Urriola, D. G. Tiffany, J.-C. Jang, G. G. Shurson, and B. Hu. 2021. Feeding Value Improvement of Corn-Ethanol Co-product and Soybean Hull by Fungal Fermentation: Fiber Degradation and Digestibility Improvement. *Food and Bioproducts Processing*. 130: 143–153. https://doi.org/10.1016/j.fbp.2021.09.013.

[12] Lee, F. H., S. Y. Wan, H. L. Foo, T. C. Loh, R. Mohamad, R. Abdul Rahim, and Z. Idrus. 2019. Comparative Study of Extracellular Proteolytic, Cellulolytic, and Hemicellulolytic Enzyme Activities and Biotransformation of Palm Kernel Cake Biomass by Lactic Acid Bacteria Isolated from Malaysian Foods. *International Journal of Molecular Sciences*. 20(20): 4979. https://doi.org/10.3390/ijms20204979.

[13] Zeng, J., W. Huang, X. Tian, X. Hu, and Z. Wu. 2021. Brewer's Spent Grain Fermentation Improves Its Soluble Sugar and Protein as Well as Enzymatic Activities Using *Bacillus velezensis*. *Process Biochemistry*. 111: 12–20. https://doi.org/10.1016/j.procbio.2021.10.016.

[14] Siddiquee, S., S. N. Shafawati, and L. Naher. 2017. Effective Composting of Empty Fruit Bunches Using Potential *Trichoderma* Strains. *Biotechnology Reports*. 13: 1–7. https://doi.org/10.1016/j.btre.2016.11.001.

[15] Arsy, S. L., A. Oetari, and W. Sjamsuridzal. 2020. Solid-State Fermentation of Sterile Slurry and Palm Kernel Cake (PKC) Mixture Using *Rhizopus azygosporus* UICC 539. *IOP Conference Series: Earth and Environmental Science*. 483(1): 012029. https://doi.org/10.1088/1755-1315/483/1/012029.

[16] Horwitz, W., and G. Latimer. 1975. *Official Methods of Analysis*. Vol. 222. Washington, DC: Association of Official Analytical Chemists.

[17] Alshelmani, M. I., T. C. Loh, H. L. Foo, W. H. Lau, and A. Q. Sazili. 2014. Biodegradation of Palm Kernel Cake by Cellulolytic and Hemicellulolytic Bacterial Cultures through Solid-State Fermentation. *The Scientific World Journal*. 2014: 729852. https://doi.org/10.1155/2014/729852.

[18] Miller, G. L. 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*. 31(3): 426–428. https://doi.org/10.1021/ac60147a030.

[19] Goering, H. K., and P. J. Van Soest. 1970. *Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications)*. Agriculture Handbook No. 379. Washington, DC: Agricultural Research Service, U.S. Department of Agriculture.

[20] Habtewold, J. Z., B. L. Helgason, S. F. Yanni, H. H. Janzen, B. H. Ellert, and E. G. Gregorich. 2020. Litter Composition Has Stronger Influence on the Structure of Soil Fungal than Bacterial Communities. *European Journal of Soil Biology*. 98: 103190. https://doi.org/10.1016/j.ejsobi.2020.103190.

[21] Patel, A. K., C.-D. Dong, C.-W. Chen, A. Pandey, and R. R. Singhania. 2023. Production, Purification, and Application of Microbial Enzymes. In *Biotechnology of Microbial Enzymes*, 2nd ed., edited by G. Brahmachari, 25–57. Academic Press. https://doi.org/10.1016/B978-0-443-19059-9.00019-0.

[22] Lateef, A., J. K. Oloke, E. B. Gueguim Kana, S. O. Oyeniyi, O. R. Onifade, A. O. Oyeleye, O. C. Oladosu, and A. O. Oyelami. 2008. Improving the Quality of Agro-Wastes by

Solid-State Fermentation: Enhanced Antioxidant Activities and Nutritional Qualities. *World Journal of Microbiology and Biotechnology*. 24(10): 2369–2374. https://doi.org/10.1007/s11274-008-9749-8.

[23] Antia, U. E., N. U. Stephen, A. A. Onilude, I.-O. M. Udo, and T. J. Amande. 2023. Bioconvertibility of Mannan-Containing Polysaccharides to Bioethanol: A Comparative Study of Palm Kernel Cake and Copra Meal Feedstocks. *Biomass Conversion and Biorefinery*. 13(6): 5175–5186. https://doi.org/10.13140/RG.2.2.11971.53289.

[24] Weimer, P. J. 2022. Degradation of Cellulose and Hemicellulose by Ruminal Microorganisms. *Microorganisms.* 10(12): 2345. https://doi.org/10.3390/microorganisms10122345.

[25] Charalampopoulos, D., S. S. Pandiella, and C. Webb. 2002. Growth Studies of Potentially Probiotic Lactic Acid Bacteria in Cereal-Based Substrates. *Journal of Applied Microbiology*. 92(5): 851–859. https://doi.org/10.1046/j.1365-2672.2002.01592.x.

[26] Iluyemi, F. B., M. M. Hanafi, O. Radziah, and M. S. Kamarudin. 2006. Fungal Solid-State Culture of Palm Kernel Cake. *Bioresource Technology*. 97(3): 477–482. https://doi.org/10.1016/j.biortech.2005.03.005.

[27] Tse, T. J., D. J. Wiens, and M. J. T. Reaney. 2021. Production of Bioethanol—A Review of Factors Affecting Ethanol Yield. *Fermentation*. 7(4): 268. https://doi.org/10.3390/fermentation7040268.

[28] Ng, T. K., and J. G. Zeikus. 1981. Comparison of Extracellular Cellulase Activities of *Clostridium thermocellum* LQRI and *Trichoderma reesei* QM9414. *Applied and Environmental Microbiology*. 42(2): 231–240. https://doi.org/10.1128/aem.42.2.231-240.1981.

[29] Fasuyi, A., S. Abiodun, and O. J. A. Akomolafe. 2014. Bioconversion and Enzymes Fortification of Palm Kernel Meal as Protein Supplement in Broiler Rations. *American Journal of Experimental Agriculture*. 4(7): 767–781. https://doi.org/10.9734/AJEA/2014/6893.

[30] Sabu, A., A. Pandey, M. Jaafar Daud, and G. Szakacs. 2005. Tamarind Seed Powder and Palm Kernel Cake: Two Novel Agro Residues for the Production of Tannase under Solid-State Fermentation by *Aspergillus niger* ATCC 16620. *Bioresource Technology*. 96(11): 1223–1228. https://doi.org/10.1016/j.biortech.2004.11.002.

[31] Prabaningtyas, R. K., D. N. Putri, T. S. Utami, and H. Hermansyah. 2018. Production of Immobilized Extracellular Lipase from *Aspergillus niger* by Solid-State Fermentation Method Using Palm Kernel Cake, Soybean Meal, and Coir Pith as the Substrate. *Energy Procedia*. 153: 242–247. https://doi.org/10.1016/j.egypro.2018.10.010.