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| RESEARCH ARTICLE

Neurospora crassa Improves Nutrient Content of Rice Bran through Solid-State Fermentation

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| ABSTRACT

Rice bran, a by-product of rice milling is abundant and nutritionally promising but limited in direct use as animal feed due to its high fiber content, low protein levels, and anti-nutritional factors. This study evaluated the effects of solid-state fermentation (SSF) with Neurospora crassa on the nutrient composition of rice bran over a 15-day period. Rice bran was inoculated with N. crassa and analyzed at 0, 5, 10, and 15 days for crude protein, ether extract, crude fiber, and ash content using AOAC methods. A T-test was performed to determine the statistical significance of changes in nutrient composition. Results showed a significant ($P<0.01$) and progressive increase in crude protein (from 12.5% to 19.68%) and ether extract (from 6.26% to 12.88%) over the fermentation period. Conversely, crude fiber content decreased markedly (from 26.55% to 12.34%), indicating effective enzymatic degradation of complex carbohydrates. Ash content remained stable, suggesting the mineral profile was unaffected by fermentation. These findings demonstrate that SSF with N. crassa can substantially enrich the nutritional value of rice bran, making it a more suitable and valuable ingredient for animal feed applications.

| KEYWORDS

Byproducts, Crude fiber, Neurospora, Fermented

| ARTICLE INFORMATION

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1. Introduction

Rice (Oryza sativa) is a staple food in Southeast Asia, specifically in the Philippines. This commodity is often subjected to milling to remove the husk and produce a by-product such as rice bran, constituting approximately 8-12% of the total rice grain (Manzoor et al., 2023). It is widely available and has potential as an ingredient for animal feed due to its protein content, lipids, vitamins, and minerals. However, despite its availability, rice bran has low overall nutritional value, which limits its direct use in animal diets. It contains high fiber levels, low protein content, and anti-nutritional factors such as phytates and trypsin inhibitors, which reduce nutrient bioavailability and digestibility (Manzoor et al., 2019). These factors contribute to poor feed efficiency and limited application in animal nutrition.

To address these nutritional limitations, various biotechnological approaches have been explored, including solid-state fermentation (SSF) using fungi. SSF is an effective method for enhancing the nutrient composition of agricultural by-products by utilizing microbial enzymatic activities. Fungal fermentation has been shown to degrade anti-nutritional factors, improve protein content, and increase digestibility, making rice bran more suitable for animal feed (Wang et al., 2023)

Neurospora crassa, a filamentous fungus, has gained attention in SSF due to its ability to produce hydrolytic enzymes such as cellulases and proteases, which help break down complex carbohydrates and proteins. It is classified as Generally Recognized as Safe (GRAS) (FDA, 2025), making it a candidate for improving rice bran's nutritional profile. Studies have demonstrated that *Neurospora crassa* fermentation can enhance protein content, reduce fiber levels, and improve amino acid composition in various agro-industrial residues (Liu et al., 2016).

Despite the potential benefits of solid-state fermentation, research on the application of *Neurospora crassa* in enhancing the nutritional value of rice bran remains limited. Understanding its effects on nutrient composition and digestibility could provide valuable insights into optimizing rice bran as a feed ingredient. This study seeks to address this research gap by assessing the changes in nutrient composition of rice bran subjected to solid-state fermentation with *Neurospora crassa*

2. Material and Methods

2.1 Rice bran

Rice bran D1 was obtained from a rice mill in Aglayan, Malaybalay City, Bukidnon, Philippines. The rice bran was ground using a cyclotec laboratory mill (CT293 Cyclotec^{TM}) to obtain the fineness and uniformity of the rice bran. Rice bran is then subjected to crude protein analysis using the Official Analytical Collaboration (AOAC) 2001.11 method for crude protein comparison with *N. crassa*

2.2. Inoculum preparation and N. crassa biomass analysis

Neurospora crassa was obtained at the College of Agriculture, Animal Nutrition Laboratory, Central Mindanao University where the species of starter culture was previously analyzed at the Macrogen laboratory, Teheran-ro, Gangnam-gu, Seoul, Republic of Korea. A small amount of the spore was spread on the surface of potato dextrose agar (PDA) incubated at 30°C for 8 days and scraped to obtain the biomass and spore for crude protein analysis and substrate inoculation.

2.3. Solid-state fermentation

For the solid-state fermentation (SSF) process, rice bran was sterilized in an autoclave at 121°C for 30 minutes to eliminate pathogens. The sterilized rice bran was weighed at 60grams and inoculated with 3 grams of *Neurospora crassa* biomass inside the flask and mixed homogeneously by stirring with a sterile spatula. After thorough mixing, each aluminum tray was filled with 20grams of inuculated substrate and incubated at 30°C for 15 days. Samples of 5 grams each were collected 5, 10, and 15 days for nutrient analysis.

2.4. Nutrient analysis

Crude protein content was analyzed according to the AOAC 2001.11 method. Sample digestion was performed using Digestor™ 2508 and Digestor™ 2520, while distillation was carried out with the Kjeltec™ 8100 system. The resulting distillates were then titrated using standardized 0.09983N hydrochloric acid for quantification.

AOAC 978.10 method is used for crude fiber analysis where refluxing condenser (Labconco Crude Fiber Digestor; 115 VAC, 50/60 Hz, 18.3 A) was used for digestion to obtain indigestible plant material while Ash is determined using AOAC 942.05 method where Muffle furnace (Thermolyne 5.8L B1) is used for combustion or burning at 700°C. In determining the Ether extract of samples, the AOAC 2003.5 method is followed using the Soxhlet apparatus (Soxtec^{TM} 8000) for boiling, rinsing, and solvent sample recovery.

2.5 Statistical Analysis

The obtained data was subjected to a t-test using R studio to determine significant differences between the fermented and non-fermented rice bran. Graphs are generated to visualize the trends in the proximate composition of fermented rice bran in different fermentation periods.

3. Results and Discussion

3.1 Crude protein contribution of N.crassa to rice bran

Figure 1 presents a comparative analysis of crude protein content between unfermented rice bran and *Neurospora crassa* biomass alone. The crude protein content of unfermented rice bran was 12.5%, whereas *N. crassa* alone exhibited a lower value of only 0.16%. This difference demonstrates that the fungal biomass itself contributes minimally to the overall protein content.

The significance of this finding lies in its implication for interpreting the results of solid-state fermentation. When rice bran is subjected to fermentation with *N. crassa*, any observed increase in crude protein content cannot be attributed to the direct addition of fungal biomass, since *N. crassa* alone contains negligible protein.

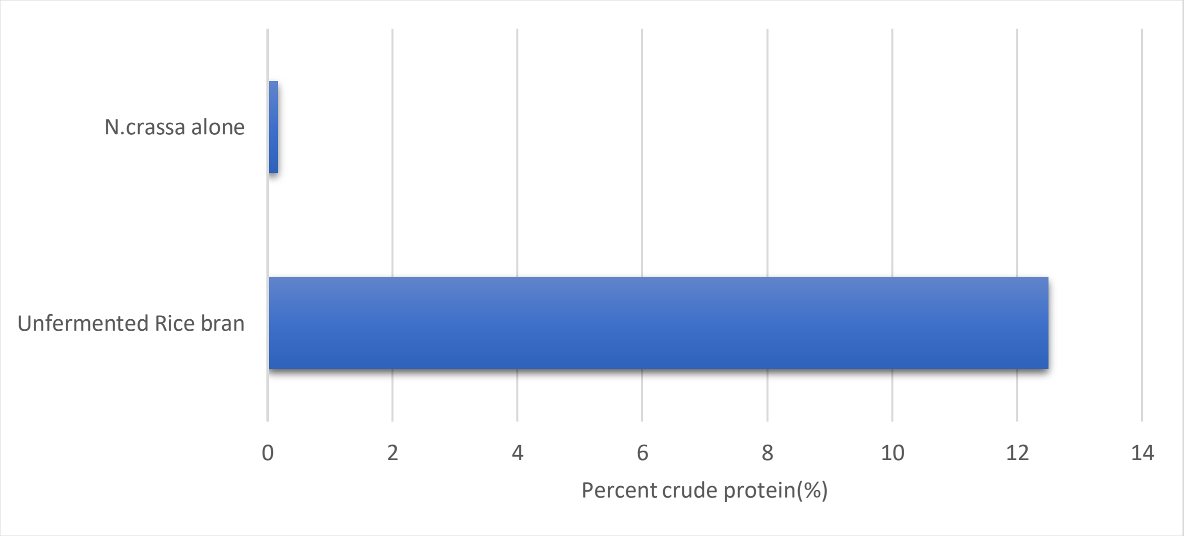

Figure 1. Crude protein comparison of between unfermented rice bran and Neurospora crassa biomass alone

3.2 Crude protein analysis

Figure 2 showed a progressive change in the nutrient composition of rice bran during solid-state fermentation with *Neurospora crassa* over a 15-day period. The crude protein of fermented rice bran significantly increased (P<0.01) (Table1) from 12.5% at 0 day to 14.54%, 16.6%, and 19.68% at days 5, 10, and 15, respectively. This progressive increase highlights the ability of *Neurospora crassa* to enhance the protein content of rice bran through solid-state fermentation. Such enhancement is primarily attributed to enzymatic degradation of complex carbohydrates and anti-nutritional factors (Huang et al., 2019) thereby concentrating the protein fraction.

3.3 Ether extract

Ether extract, often called crude fat, represents the lipid content of a sample, also showed a significant (P<0.01) and progressive increase over time, rising from 6.26% to 7.28%, 9.34 and 12.88% at day 5, 10, 15, respectively (Figure 2, Table 1). This increase in lipid content can enhance the energy density of the fermented rice bran, making it a more valuable feed ingredient for animals requiring higher energy intake (Abaspour et al., 2024).

The enrichment of fat content during fermentation is likely due to the accumulation of fungal lipids and the breakdown of complex carbohydrates, which may concentrate the lipid fraction (Oleveira et al., 2011). Additionally, some fungi are known to synthesize lipids as part of their metabolic processes during growth, which contributes to the overall increase in ether extract (Zhang et al., 2022).

However, the elevated fat content also poses potential challenges. Lipids, particularly unsaturated fatty acids, are susceptible to oxidation, leading to lipid rancidity, off-flavors, and quality deterioration during storage (Shahid & Hosain., 2022).

3.4 Crude fiber

Crude fiber is a measure of indigestible plant cell wall components, primarily cellulose and lignin, that remain after a plant food has been treated with dilute acid and alkali in a laboratory (Cherian, 2019). the crude fiber content exhibited a pronounced and statistically significant (P<0.01) downward trend (Table 1, Figure 2), decreasing from to 26.55% 22.41%, 16.49%, and 12.34% at days 5, 10, and 15, respectively. This marked reduction in fiber content suggests effective enzymatic degradation of complex carbohydrates by *N. crassa*, which can enhance the digestibility and palatability of rice bran for animal feed applications. The enzymatic breakdown of fiber components is primarily attributed to the production of cellulases, hemicellulases, and lignin-degrading enzymes by *N. crassa* (Verma et al., 2020). These enzymes hydrolyze the complex polysaccharides and lignin structures, reducing the fiber content and consequently improving the digestibility and palatability of rice bran when used as animal feed (Liu et al., 2021).

3.5 Ash

Ash content, which reflects the total mineral content of rice bran, remained relatively stable throughout the fermentation process, with values fluctuating only slightly between 12.91% and 12.67%, and no statistically significant differences observed (Table 1, Figure 2). This stability indicates that the mineral profile of rice bran is not affected during fermentation.

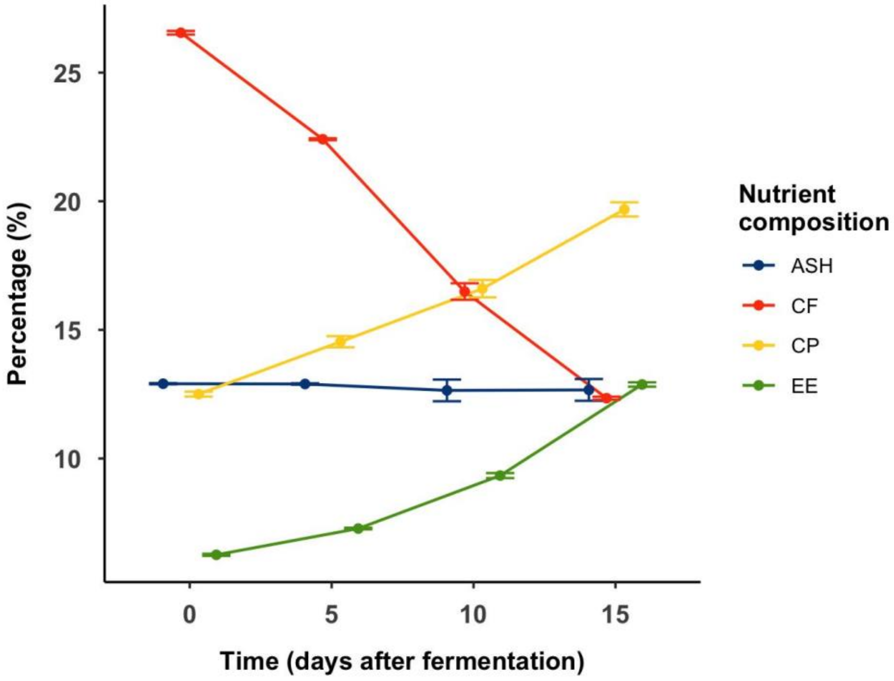

Figure 2. Nutrient composition of fermented rice bran over-15-day period

Table 1. *P* value from the T-test of different nutrient composition of fermented and unfermented rice bran

Nutrient composition	FERMENTATION TIME		
	5	10	15
CP			
0	0.001018**	0.001151**	0.000128**
ASH			
0	0.639^{ns}	0.3973^{ns}	0.429^{ns}
CF			
0	3.01E-06**	0.000191**	1.76E-09**
EE			
0	8.05E-06**	5.05E-05**	2.12E-06**

**- highly Significant

^{ns}- non-significant

0 – unfermented rice bran

4. Conclusion

Solid-state fermentation of rice bran with *Neurospora crassa* resulted in a time-dependent improvement in its nutritional profile, characterized by significant increases in crude protein and ether extract, and a reduction in crude fiber content, while maintaining stable mineral levels. The observed enhancements are attributed to the enzymatic activities of *N. crassa*, which effectively degrade complex carbohydrates and concentrate protein and lipid fractions. These results highlight the potential of SSF as an effective biotechnological approach to upgrade rice bran for animal feed use. However, the increased fat content warrants careful consideration of storage and handling to prevent lipid rancidity. *N. crassa* fermentation offers a promising strategy for rice bran nutrient enrichment and improving feed efficiency in animal production systems.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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