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Effect of Incubation Period on the Physical Quality, pH, and Bulk Density of Fermented Rice Bran

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Abstract: This study evaluated the effect of incubation period on the physical quality of fermented rice bran prepared with an EM4-based starter under anaerobic conditions. Rice bran was assigned to four incubation periods, namely 0 day (P0), 7 days (P1), 14 days (P2), and 21 days (P3). The observed variables were color, aroma, texture, clumping tendency, insect-related visual score, pH, and bulk density. Incubation period modified several physical attributes of the material. Color scores decreased after fermentation, indicating a shift from brown to more yellowish-brown material, whereas aroma scores improved as incubation lengthened. Texture remained relatively unchanged across treatments. In contrast, the clumping score declined with longer incubation, showing a greater tendency of the product to form aggregates. The insect-related visual score also decreased after fermentation. The pH value declined progressively from the non-incubated treatment to the 21-day treatment, indicating increasing acidification during fermentation. Bulk density increased numerically up to 14 days and then slightly decreased at 21 days, although values remained higher than the control. Overall, incubation period was an important determinant of fermented rice bran quality. Longer incubation enhanced aroma and acid development, but it also altered color and increased clumping, indicating that incubation time should be selected according to the desired balance between sensory improvement and physical handling characteristics.

Keywords: Rice bran, Fermentation, Incubation period, Physical characteristic

Introduction

Rice bran is one of the most abundant by-products generated during rice milling and has long been used as an energy source in livestock feeding. Its practical value, however, is often constrained by rapid deterioration after milling, variable physical quality, and the development of undesirable odors during storage. Rice bran contains active lipolytic and oxidative enzymes, so rancidity can develop quickly when the material is exposed to moisture, oxygen, and fluctuating temperature conditions. Gao et al. (2021) reported that storage increased aldehydes, ketones, and alcohols associated with early oxidation in rice bran, while Wu et al. (2020) demonstrated that rancidity can alter the oxidation status and structure of rice bran protein. These characteristics are important in feed practice because the physical condition of the ingredient, including color, odor, clumping tendency, and freedom from visible contamination, influences not only acceptability but also storage stability, handling, and mixing uniformity. Thus, efforts to improve rice bran utilization should not focus exclusively on nutrient composition; they should also consider the physical attributes that determine whether the material remains acceptable as a feed ingredient.

Fermentation has become one of the most widely studied approaches for upgrading agricultural by-products, including rice bran. Through the action of beneficial microorganisms, fermentation can acidify the substrate, hydrolyze structural carbohydrates, alter volatile compounds, and increase the availability of valuable nutrients or bioactive constituents. Several recent studies have shown that fermented rice bran may possess better functional or nutritional characteristics than the unfermented material. Moon and Chang (2021) showed that rice bran fermented with Lactiplantibacillus plantarum could be developed as a functional material with improved biochemical properties. Punia et al. (2021) reported that Aspergillus oryzae fermentation enhanced bioactive compounds and antioxidant potential in rice bran, while Liu et al. (2022) found that mixed-bacteria solid-state fermentation improved antioxidant activity and increased several bioactive components. Omarini et al. (2019) also demonstrated that solid-state fermentation upgraded the nutritional value of rice bran. In animal feeding contexts, fermented rice bran has also been associated with improved digestibility and biological responses. Dilaga et al. (2022) found that inoculant type and dose influenced the nutritional quality and in vitro digestibility of fermented rice bran, whereas Wang et al. (2023) observed that fermented heat-treated rice bran improved nutrient digestibility and positively influenced intestinal conditions in laying hens. Likewise, Huervana et al. (2024) showed that solid-state fermentation converted rice bran into a higher-protein feed ingredient for shrimp.

Besides improving nutrient availability, fermentation also modifies organoleptic and physicochemical characteristics. Astuti et al. (2022) demonstrated that solid-state fermentation changed the volatile compound profile and aroma description of rice bran, while Wang et al. (2025) observed that fermentation by yeast generated a more diverse and favorable aroma profile. Changes in acidity are also central to the fermentation process because acid production reflects microbial activity and can help suppress undesirable spoilage organisms. Manlapig et al. (2023) reported that lactic acid bacteria fermentation altered the organic acid profile of rice bran, and Manlapig et al. (2024) further showed that fermented rice bran could modify in vitro fermentation characteristics. Structural changes induced by fermentation may also affect the way a material behaves physically, including its compactness, bulk density, and tendency to absorb water or form aggregates. Wu et al. (2024) found that combining solid-state fermentation and extrusion altered the quality attributes of rice bran, and Ye et al. (2025) reported that lactic acid bacteria fermentation modified the physicochemical properties of black rice bran. These findings indicate that fermentation is not only a biochemical process but also a process that reshapes the sensory and handling characteristics of bran.

Even though the recent literature has advanced understanding of the nutritional, antioxidant, and functional properties of fermented rice bran, fewer studies have focused specifically on simple physical quality parameters that are highly relevant in routine feed evaluation. In many practical settings, farmers and feed handlers first judge a fermented ingredient by visible color, odor, tactile texture, clumping behavior, and apparent cleanliness before conducting laboratory analyses. Basic measurements such as pH and bulk density are also valuable because they provide rapid information about fermentation progress and the expected behavior of the ingredient during storage, transport, and feed mixing. However, many published studies emphasize inoculant type, nutrient composition, or biological performance, whereas the effect of incubation period on straightforward physical quality indicators remains less clearly documented, especially under simple anaerobic fermentation conditions commonly used at farm or laboratory scale. Incubation time is a crucial variable because overly short fermentation may not allow sufficient acidification, while excessively long incubation may worsen physical deterioration, promote clumping, or reduce visual quality.

The present study was therefore designed to evaluate the effect of incubation period on the physical quality of fermented rice bran. Four incubation periods were compared: 0, 7, 14, and 21 days. The evaluated responses included organoleptic parameters (color, aroma, texture, clumping tendency, and insect-related visual score), pH, and bulk density. The study is expected to contribute practical information for determining how long rice bran should be incubated to obtain acceptable physical characteristics under anaerobic fermentation conditions.

Materials and Methods

This study was conducted at the Laboratory of Animal Feed Nutrition and Technology, Department of Animal Husbandry, Faculty of Agriculture, University of Lampung, from January to February 2025. The experiment was conducted in a laboratory-scale setting using rice bran as the fermentation substrate. The manuscript was prepared from the original research result file and the accompanying analytical procedure file supplied by the author. The main materials were rice bran, EM4 as the microbial starter, sugar, water, plastic bags for anaerobic incubation, a digital pH meter, and a measuring cylinder for bulk density determination.

The experiment consisted of four treatments based on incubation period: P0 = 0 day, P1 = 7 days, P2 = 14 days, and P3 = 21 days. Rice bran was weighed at 1 kg per experimental unit. Prior to fermentation, EM4 was applied at a concentration of 20% and diluted with water before mixing with the substrate. Granulated sugar was added at 1% (w/w), equivalent to 10 g per 1 kg of rice bran, as an additional fermentable carbohydrate source to support microbial activity during fermentation. The substrate was then mixed thoroughly, packed tightly into plastic bags under anaerobic conditions, pressed to minimize entrapped air, and sealed securely. The packed material was incubated according to each treatment period. At the end of each incubation period, the bags were opened and the fermented rice bran was evaluated.

Physical quality evaluation was carried out organoleptically using visual, olfactory, and tactile observations. The evaluated parameters were color, aroma, texture, clumping tendency, and insect-related visual score. Scoring followed a five-point scale derived from the source procedure. For color, scores ranged from 5 (dark brown) to 1 (very less brown). For aroma, the scoring was adjusted to reflect fermentation characteristics, ranging from 5 (fresh acidic aroma, typical of well-fermented rice bran) to 1 (typical aroma of non-fermented rice bran). Intermediate scores were assigned as follows: 4 = slightly acidic and fairly fresh, 3 = neutral or less specific aroma, and 2 = weak fermented aroma tending toward the original bran odor. For texture, scores ranged from 5 (very fine) to 1 (very coarse). For clumping tendency, scores ranged from 5 (very crumbly or no clumps) to 1 (heavily clumped). For the insect parameter, scores ranged from 5 (no insects) to 1 (insects over the entire surface).

The pH value was measured using a digital pH meter. Bulk density was determined by pouring the sample into a 100 mL graduated cylinder and recording its weight. Data are presented as mean ± standard deviation. Organoleptic data were analyzed using one-way analysis of variance (ANOVA), and significant differences among treatment means were separated using Duncan's multiple range test at $P<0.05$. Different superscripts within the same column indicate significant differences. Because the source dataset for pH and bulk density was available only as mean ± standard deviation without post hoc grouping letters, these two variables are presented and discussed descriptively.

Results and Discussion

The incubation period clearly affected several physical quality traits of fermented rice bran (Table 1). The color score was highest in the non-incubated treatment (P0) and declined after fermentation. Based on the scoring criteria, this result indicates that the material shifted from brown toward a more yellowish-brown appearance as incubation time increased. A significant reduction in color score was already evident at 7 days and remained lower at 14 and 21 days. This change is consistent with previous reports showing that fermentation and subsequent biochemical reactions can modify rice bran pigments, oxidation products, and the overall visual character of bran materials (Astuti et al., 2022; Wu et al., 2024). Changes in bran color may result from microbial metabolism, redistribution of moisture, partial oxidation of bran constituents, and the formation of new compounds during fermentation.

Table 1. Physical quality scores of fermented rice bran at different incubation periods

Treatment	Color	Aroma	Texture	Clumping tendency	Insect score
P0	4.13 ± 0.15c	3.50 ± 0.01a	2.67 ± 0.29	4.00 ± 0.01c	3.33 ± 0.58b
P1	3.80 ± 0.10b	3.80 ± 0.20ab	2.50 ± 0.01	2.83 ± 0.29b	2.00 ± 0.01a

P2	3.50 ± 0.01a	3.87 ± 0.15b	2.50 ± 0.01	2.00 ± 0.01a	2.50 ± 0.01ab
P3	3.50 ± 0.01a	4.13 ± 0.11b	2.50 ± 0.01	2.00 ± 0.01a	2.16 ± 0.29a

Different superscripts within the same column indicate significant differences (P<0.05).

In contrast to color, aroma scores improved with longer incubation. Under the scoring system used in this study, higher values indicate a fresh acidic aroma typical of well-fermented rice bran, whereas lower values represent the characteristic odor of non-fermented bran. Therefore, the increase in aroma score from P0 to P3 indicates that fermentation progressively shifted the product from the native bran odor toward a fresher fermented aroma. This result agrees with studies showing that fermentation changes the volatile composition of rice bran and can enhance favorable aroma notes while reducing the dominance of oxidation-related volatiles (Gao et al., 2021; Astuti et al., 2022; Wang et al., 2025). In practical terms, the improved aroma likely reflected the progression of microbial fermentation and the development of acidic and fermented notes that were considered more acceptable than the initial odor of the unfermented material.

Texture scores showed only slight numerical changes among treatments and did not exhibit a clear response pattern. All treatments remained within a relatively narrow range, indicating that incubation period had limited influence on the perceived coarseness of the material. This finding suggests that fermentation under the present conditions did not fundamentally alter particle size or the tactile fineness of the bran. Even when fermentation changes biochemical properties, the original milling characteristics of rice bran may still dominate tactile perception, especially if the substrate is not remilled after incubation.

A different pattern was observed for clumping tendency. The clumping score decreased markedly from P0 to P2 and P3, indicating that prolonged incubation increased the tendency of the material to form aggregates. This response is reasonable because fermentation involves moisture redistribution and microbial activity that can increase adhesion among particles. Structural modification of bran during fermentation has been reported previously, and such changes may affect physical behavior during handling (Moon & Chang, 2021; Ye et al., 2025). From a feed management perspective, greater clumping may reduce ease of mixing and flowability, even when fermentation improves other quality traits. Therefore, the best incubation period should not be judged only from aroma improvement, but also from the resulting physical handling properties.

The insect-related visual score also differed among treatments. The non-incubated treatment showed a higher score than the fermented treatments, whereas the lowest values occurred at 7 and 21 days. Because the scoring system represented visible insect contamination, the decline in score indicates that the insect-related visual condition was less favorable in the fermented groups under this dataset. This result should be interpreted carefully, however, because the source documents did not provide additional observational detail regarding the context of insect scoring after incubation. Even so, the data indicate that incubation period influenced this practical quality attribute and should be considered when fermented rice bran is stored or handled.

The pH data in Table 2 show a progressive decline from 5.93 in P0 to 5.10 in P3. This trend indicates that acid production continued as fermentation proceeded. Such acidification is a typical sign of active microbial fermentation and has also been reported in fermented rice bran and related fermented feed materials (Moon & Chang, 2021; Manlapig et al., 2023; Wang et al., 2023). Lower pH values are generally associated with the accumulation of organic acids, especially when lactic acid bacteria are involved directly or indirectly in the fermentation consortium. From a preservation standpoint, decreasing pH is desirable up to a certain level because it may help stabilize the substrate and suppress undesirable microbes. In the present study, the gradual decline in pH supports the observation that 14 and 21 days allowed a more advanced fermentation process than 7 days. Because these pH data were available only as descriptive mean ± standard deviation values in the source dataset, the trend is interpreted numerically rather than through post hoc statistical grouping.

Table 2. pH values of fermented rice bran at different incubation periods

Treatment	pH
P0	5.93 ± 0.15
P1	5.40 ± 0.17
P2	5.23 ± 0.11
P3	5.10 ± 0.10

Table 3. Bulk density values of fermented rice bran at different incubation periods

Treatment	Bulk density
P0	294.7 ± 2.31
P1	309.3 ± 10.07
P2	317.3 ± 6.11
P3	310.7 ± 11.55

Bulk density values showed a numerical increase from P0 to P2, followed by a slight decline at P3, although the 21-day treatment still remained above the control (Table 3). Because bulk density data were available only as descriptive mean ± standard deviation values, the pattern was interpreted numerically rather than by inferential comparison. This pattern suggests that incubation modified the packing behavior of the bran particles. A moderate increase in bulk density may be related to partial structural collapse, changes in moisture distribution, or reduced inter-particle void space during fermentation. Similar evidence that fermentation modifies structural and physicochemical behavior has been reported in rice bran and bran-based matrices (Huervana et al., 2024; Wu et al., 2024; Ye et al., 2025). In feed manufacture, bulk density is important because it affects storage capacity, transportation efficiency, and homogeneity during mixing. The present result therefore indicates that incubation period not only changes the sensory properties of fermented rice bran but may also alter its engineering and handling characteristics.

Conclusion

Incubation period altered the physical quality of fermented rice bran. Extending incubation from 0 to 21 days improved aroma and lowered pH, while reducing color score and increasing clumping tendency. Texture changed little, whereas bulk density rose numerically up to 14 days. These findings show that fermentation time should be selected by balancing sensory improvement, acidity development, and physical handling characteristics.

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