

Effect of Solid-State Fermentation on Phytochemical and Proximate Composition of Millet Bran Meal

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Abstract

The majority of a fish farm's overall operating expenses involve costs related to feeds. Commercial feeds are often too expensive for fish farmers. Consequently, farmers do use agricultural by-products and locally available fish feed ingredients to improve pond productivity and minimise cost. This study was aimed at evaluating the effects of solid-state fermentation on phytochemical components and proximate composition of millet bran meal for possible incorporation in fish feed production. Millet bran was collected in three different bowls, ground to powder, and then sieved and fermented using a solid-state fermentation procedure. Each sample was fermented (wet at 10% moisture and kept in a container at ambient temperature) for 24 hours (1 day), 96 hours (4 days), and 168 hours (7 days). Afterwards, the fermented samples were sun-dried for 94 hours (4 days approximately). Quantitative phytochemical screening was carried out on the samples which were analysed for proximate composition. The results revealed that fermenting millet bran for seven days (168hrs) using solid-state fermentation method reduced the alkaloid content from 14.69 ± 1.24 to 10.12 ± 0.16 , phytate from 5.73 ± 1.32 to 3.25 ± 0.73 , tannins from 6.62 ± 0.53 to 4.62 ± 0.84 , oxalate from 17.78 ± 1.65 to 13.82 ± 0.53 . However, it increases flavonoids from 11.95 ± 1.82 to 13.08 ± 1.35 and phenols from 23.78 ± 1.65 to 31.71 ± 0.62 . The study also elucidates an increase in crude protein from 9.23 ± 0.64 to 19.77 ± 0.53 and a reduction in crude fibre from 7.41 ± 0.52 to 6.02 ± 1.14 . This study demonstrates that solid-state fermented millet bran meal can be incorporated as an essential part of feed production.

Keywords: Millet Bran Meal, Solid-State Fermentation, Phytochemical Screening, Proximate Composition Analysis.

Introduction

Fish farming is often faced with high feed cost comprising approximately 60 to 75% of total production costs; these causes decrease in profit margin for the fish farmers (Mohammed *et al.*, 2017). An attempt to upsurge the profit is to minimize the cost of feed as minimal as possible. One way to do this is to explore local feed ingredients that could be used as

alternatives for fish feed. In selecting the local feed ingredients, several factors should be considered. Amongst others are the availability and accessibility of the ingredients, the cost, the nutrients, and the extent of its competition as a source of food with humans (Handajani and Widodo, 2010; Nwafor *et al.*, 2023). However, problem of high cost of feeding in aquaculture is further exacerbated due to the scarce and expensive nature of some of the ingredients used in the formulation of fish feeds. In other to solve the problem of scarcity and expensiveness of feed ingredients, a number of non-conventional feedstuffs have been investigated most of which are alternative nutrient sources (Sogbesan and Ugwumba, 2008; Yafetto, 2022).

Among the alternative local feed ingredients that can be used to feed catfish is fermented millet bran which is considered as a by-product. Millet is one of the cereals besides the wheat, rice, and maize. Millets are major food sources for millions of people, especially those who live in hot, dry areas of the world. They are grown mostly in marginal areas under agricultural conditions in which major cereals fail to give substantial yields (Adekunle, 2012). Millets are classified with maize, sorghum, and Coix (Job's tears) in the grass sub-family *Panicoideae* (Yang *et al.*, 2012). Millets are important foods in many countries because of their ability to grow under adverse weather conditions like limited rainfall.

Solid-state fermentation is a microbial fermentation bioprocess involving cultivation of microorganisms on a moist, solid, non-soluble organic material that provides nutrient source for the growth of the microorganisms, in the full or partial absence of free-flowing water (Manan and Webb, 2017). Although there are varying steps involved in solid-state fermentation that are widely used in industry but there are slight differences in the approaches dependent on the final desired product (Yafetto, 2022). Slightly modified approaches for solid-state fermentation studies that were aimed at enriching the protein contents of grains and cassava peels were used (Nambi *et al.*, 2017; Yafetto *et al.*, 2019 and 2020). According to Selo *et al.* (2021) and Wang *et al.* (2022) the differences in the approaches for solid-state fermentation are technologically feasible across the board at different stages of bioprocessing, both at the laboratory level and on pilot scales.

This research is aimed at exploring bioprocessing of millet bran using solid-state fermentation method with varying duration in order to evaluate the effects on the nutritional value of the millet bran by carrying out phytochemical screening and proximate analysis on the bioprocessed millet bran.

Materials and Methods

Experimental Site

The experiment was carried out at the Teaching and Research Fish Farm of the Department of Fisheries Technology, School of Sciences, Yobe State College of Agriculture, Science and Technology Gujba, Yobe State, Nigeria; located at 5km Gujba road Damaturu, situated at longitude 11.678°N and latitude 11.946°E.

Source of Experimental Ingredient

Millet bran was procured from millet milling in Damaturu, Damaturu Local Government Area of Yobe State, Nigeria.

Processing of Millet Bran

Millet bran was collected in three different bowls, ground into powder, then sieved and fermented using solid-state fermentation procedure as described by Sogbesan *et al.* (2012). Each sample was solidly fermented (wet at 10% moisture and kept in container at ambient temperature) for 24 hours (1 day), 96 hours (4 days), and 168 hours (7 days). Afterward, the fermented samples were sun dried for 94 hours (4 days approximately).

Phytochemical Screening of Fermented Millet Bran Meal

Two (2) grams each of the samples collected were used for quantitative phytochemical screening to assess alkaloid, flavonoids, phenols, phytate, oxalate, saponin and tannins on each of the treatments following the methods of Association of Official Analytical Chemist Methods (AOAC, 1984) and Edeoga *et al.* (2005).

Proximate Analysis of the Fermented Millet Bran Meal

The fermented Millet bran at a various solid-state fermentation period were analysed for crude protein, crude fibre, crude lipid, ash and a nitrogen free extract according to A.O.A.C. (2010).

Statistical Analysis

All data generated were subjected to descriptive statistics to determine the mean values and then subjected to analysis of variance (ANOVA) at 95% probability level where the significant differences were detected. Mean values were separated using Least Significant Difference (LSD). All data were analysed using SPSS (Statistical Package for Social Sciences) version 20.0.

Results

Phytochemical Composition of the Solid Fermented Millet Bran Meal

The quantitative investigation of the phytochemical constituents of the solid-state fermented Millet bran at various fermentation period shows that Alkaloid, Flavonoids, Phenols, Phytate, Oxalate, Saponin and Tannin are present in all the fermented bran meals at 24 hours (1 day), 96 hours (4 days) and 168 hours (7 days) of fermentation as elucidated in Table 1. The highest content of Alkaloids in the solid-state fermented Millet bran was recorded in 0 hour (0 day) fermentation with 14.69 ± 1.24 mg/kg and the lowest was recorded in 168 hours (7 day) fermentation with 10.12 ± 0.16 . There is no significance ($P < 0.05$) between 0 hour and day one. However, significant difference ($P < 0.05$) was observed between day one and day seven. The highest content of Flavonoids was recorded in 168 hours (7 days) fermentation with 13.08 ± 1.35 mgRutin/kg and the lowest was recorded in 24

hours (1 day) fermentation with 11.90 ± 1.65 mgRutin/kg. There was no significant difference ($P < 0.05$) between 0 hour and day one. However, there was significant difference ($P < 0.05$) between 0 hour and day seven. The highest content of Phytate, Tannins, Saponins and oxalate in Millet bran were recorded in 0 hours (0 day) fermentation with 5.73 ± 1.32 mg/kg, 6.62 ± 0.53 mgAT/kg, 2.98 ± 0.54 g/kg and 17.78 ± 1.65 mg/100g respectively while the lowest were recorded in 168 hours (7 days) fermentation with 3.25 ± 0.73 mg/kg, 4.62 ± 0.84 mgAT/kg, 1.43 ± 1.17 g/kg and 13.82 ± 0.53 mg/100g respectively and highest content of Phenols was recorded in 168 hours (7 day) fermentation with 31.71 ± 0.62 GAE/kg and the lowest was recorded in 0 hour (0 days) fermentation with 23.78 ± 1.65 . There was significant difference ($P < 0.05$) between day one and day seven.

Table 1: Quantitative Phytochemical Composition of Solid-State Fermented Millet Bran Meal

Parameters	0 Day Fermentation	1 Day Fermentation	4 Days Fermentation	7 Days Fermentation
Alkaloids (mg/kg)	14.69 ± 1.24^a	14.24 ± 1.52^a	13.18 ± 0.12^b	10.12 ± 0.16^c
Flavonoids (mgRutin/kg)	11.95 ± 1.82^c	11.90 ± 1.65^c	12.03 ± 1.43^b	13.08 ± 1.35^a
Phytate (mg/kg)	5.73 ± 1.32^a	4.39 ± 0.82^c	5.05 ± 0.56^b	3.25 ± 0.73^d
Tannins (mgTA/kg)	6.62 ± 0.53^a	6.11 ± 0.65^a	6.25 ± 0.27^a	4.62 ± 0.84^b
Saponins (g/kg)	2.98 ± 0.54^a	2.59 ± 0.17^a	2.88 ± 1.54^a	1.43 ± 1.17^b
Phenols (gGAE/kg)	23.78 ± 1.65^c	30.91 ± 1.83^b	31.14 ± 0.12^a	31.71 ± 0.62^a
Oxalate (mg/100g)	17.78 ± 1.65^a	17.47 ± 1.48^a	16.91 ± 0.32^a	13.82 ± 0.53^b

Means in the Same Row with Different Superscripts are Significantly Different ($P < 0.05$)

Proximate Analysis Composition of the Solid-state Fermented Millet Bran

Table 2 elucidates the proximate composition of the feed ingredient. The highest percentage moisture and crude lipid content in the fermented Millet bran recorded were highest in 168 hours (7days) fermentation with 17.20 ± 0.68 and 10.98 ± 1.54 and the lowest were found in 0 hour (0 day) fermentation with 11.00 ± 0.53 and 6.35 ± 0.82 respectively. The highest percentage dry matter content recorded was highest in 0 hours (0 day) fermentation with 89.00 ± 1.05 and the lowest was recorded in 168 hours (7 days) fermentation with 82.80 ± 1.36 . The percentage ash content was highest in 24 hours (1 day) fermentation with 7.71 ± 0.15 and the lowest was recorded in 0 hour (0 days) fermentation with 5.20 ± 1.53 while the percentage crude fibre recorded was highest in 0 hour (0 day) fermentation with 7.41 ± 0.52 and the lowest was recorded in 168 hours (7 days) fermentation with 6.02 ± 1.14 . The highest percentage crude protein was recorded in 96 hours (4 days) with 19.78 ± 0.45 and the lowest was recorded in 0 hour (0 day) fermentation with 9.23 ± 0.64 . The percentage nitrogen free extract (NFE) recorded was highest in 0 hour

(0 day) fermentation with 64.81 ± 1.84 and the lowest was recorded in 168 hours (7 days) fermentation with 40.39 ± 1.47 . There is a significance difference ($P < 0.05$) among the treatments.

Table 2: Proximate Composition of Solid Fermented Millet Bran Meal (g/100g)

Parameters	0 Day Fermentation	1 Day Fermentation	4 Days Fermentation	7 Days Fermentation
Moisture	11.00 ± 0.53^c	11.65 ± 0.13^c	15.60 ± 0.28^b	17.20 ± 0.68^a
Dry Matter	89.00 ± 1.05^a	88.35 ± 1.05^a	84.40 ± 1.32^b	82.80 ± 1.36^c
Ash	5.20 ± 1.53^c	7.71 ± 0.15^a	6.10 ± 1.56^b	5.64 ± 0.23^c
Crude Lipid	6.35 ± 0.82^c	10.32 ± 1.02^b	10.43 ± 0.12^b	10.98 ± 1.54^a
Crude Fibre	7.41 ± 0.52^a	6.78 ± 0.98^a	6.17 ± 1.13^b	6.02 ± 1.14^b
Crude Protein	9.23 ± 0.64^c	13.98 ± 0.72^b	19.78 ± 0.45^a	19.77 ± 0.53^a
NFE	64.81 ± 1.84^a	49.56 ± 1.51^b	41.92 ± 0.68^c	40.39 ± 1.47^c

Means in the Same Row with Different Superscripts are Significantly Different ($P < 0.05$)

Discussion

Phytochemical Composition of Solid-State Fermented Millet Bran Meal

The phytochemical investigation of the solid-state fermented Millet bran at varying fermentation periods revealed that alkaloids, flavonoids, phenols, phytate, oxalate, saponins and tannins were present at varying concentrations as presented in the Table 1. These chemical compounds were synthesized in natural food and / or feedstuffs by the normal metabolism of the plant species as reported by Habtamu and Niegussie (2014). This result agreed with Soetan and Oyewole (2009) who reported that phytochemical compounds reduce feed intake and nutrients utilization of plants or plant products used for feeds and they play vital roles in determining the use of plants for fish feed. The results obtained from this study corroborates the findings of Habtamu and Niegussie (2014) who reported that alkaloids are one of the largest groups of chemical compounds synthesized by plants and generally found as salts of plant acids such as oxalic, malic, tartaric or citric acid. The study also corroborates with that of Gao *et al.* (2019) who reported that flavonoids are diphenylpropanes that constitute one of the most characteristic classes of secondary metabolites in plants. Flavonoids have been shown to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions and lipid peroxy radicals, and have been reported as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory actions. The study also agreed with that reported by Akande *et al.* (2010) that oxalic acid binds with nutrients, rendering them inaccessible to the body; however, with reduction in the oxalate content in the solid fermented millet bran for 198 hours, this implied that the nutrients will be accessible as there are less quantity of oxalic acid to bind with them. This study is also in line with the

report of Walter *et al.* (2002) that Phytate is also metabolites that is an inositol hexakisphosphate containing compound that binds with minerals and inhibits mineral absorption causing mineral deficiency due to its low bioavailability in the diet.

Similarly, the study is in concord with the findings of Umaru *et al.* (2007) who reported that saponins reduce the uptake of glucose and cholesterol at the gut through intra-luminal physicochemical interaction. It also agreed with that of Akande *et al.* (2010) who reported that saponins can affect animal performance and metabolism in a number of ways which include erythrocyte haemolysis, reduction of blood and liver cholesterol, depression of growth rate, enzyme inhibition and reduction in nutrient absorption. This result also agreed with Smith *et al.* (2010) who reported tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein such as digestive enzymes.

The results obtained from the proximate composition of the solid-state fermented Millet bran meal revealed that the highest percentage moisture content, crude lipid and crude protein values were found in (7 days) fermentation. The variation observed from these values often exists during the process of chemical analysis of experimental ingredients as reported by Falaye *et al.* (2014) that the lowest percentage crude lipid was recorded in seven (7) days fermentation. This result is in line with Ekpe (2019) who stated that bio-fermentation treatment was able to improve protein and reduce crude fibre of rice husk from 59.20% which was the value of crude fibre in untreated rice husk to 37.34% crude fibre. It also in support of Farizaldi *et al.* (2017) who reported that, fermentation reduced crude fibre of coconut waste from 7.66% to 6.09% using bread yeast for six days subsequently, moisture content, crude protein and crude lipid content observed were higher in diet 5 that had the highest inclusion level. This result is in agreement with findings of Daipo *et al.* (2017) which reported that fermentation improve the protein content and reduces crude fibre content of rice husk and Falaye *et al.* (2014) who reported proportional increase in crude lipid values with increase in inclusion levels of growth promoter in poultry diets.

Nitrogen Free Extract (NFE) values recorded from this study was very low compared to diets containing highest inclusion level of fermented millet bran meal as reported by Agbabiaka *et al.* (2013) that the NFE values of the treatment diets with growth promoters decreased with increasing inclusion levels compared with that of the control diet. This is also in agreement with the findings of Murthy *et al.* (2016) who reported a reduction in the NFE values of fish diet with growth promoters. Similarly, Farizaldi *et al.* (2017) reported decrease in NFE in the diet of *Clarias gariepinus* fed fermented coconut waste at varying inclusion levels.

Conclusion and Recommendation

This study has revealed that solid-state fermentation of millet bran meal for seven days fermentation period resulted in desirable nutritional values both in phytochemical

constituents and proximate composition. Therefore, solid-state fermented millet bran meal is recommended for incorporation in fish feed production.

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