Indian J. Fish., 69(3): 161-166, 2022
DOI: 10.21077/ijf.2022.69.3.120851-20

Note

Effect of fungal fermentation on proximate composition, physical and functional properties of five different plant proteins used in aquafeed formulation

R. JANNATHULLA AND J. SYAMA DAYAL
ICAR-Central Institute of Brackishwater Aquaculture, 75, Santhome High Road, RA Puram, Chennai - 600 028 Tamil Nadu, India
e-mail: rjannathulla@gmail.com

ABSTRACT

Five different plant proteins such as soybean meal (SBM), groundnut oil cake (GNC), rapeseed meal (RSM), sunflower oil cake (SFC) and guar meal (GRM) were tested for proximate composition as well as physical and functional properties before and after fermentation using *Aspergillus niger.* Fermentation was carried out in a BOD incubator at 35°C for three days after inoculating with 5% fungal suspension. Results revealed that fermentation significantly ($p<0.05$) increased the crude protein content and total ash by 12.5 and 10.5%, respectively, while other proximate indices, including crude fat, crude fiber and nitrogen-free extract reduced significantly. The fungal fermentation had no effect on the texture and colour of plant proteins, while fermented samples had slightly fermented odour along with their natural odour. The plant proteins such as SBM, GNC, RSM, SFC and GRM had bulk densities of 0.54, 0.62, 0.79, 0.60 and 0.71 g cm^{-3} which significantly ($p<0.05$) increased by 27.1, 0.5, 0.4, 19.9 and 12.3%, respectively. Fermentation significantly ($p<0.05$) reduced water holding capacity by 11.7% regardless of the ingredients, with the decrease being significantly ($p<0.05$) greater in GNC (17.7%), RSM (16.4%) and SBM (13.4%). After fermentation, the protein solubility index reduced significantly ($p<0.05$) from 77.12 to 73.59%, with SBM and RSM having a reduction of about 5.9 and 5.4% respectively, while GNC and SFC had a reduction of 4%. The difference between fermented and unfermented GRM on the other hand, was not significant. Results concluded that fermented ingredients could be potential protein sources rather than their counterparts based on their chemical composition and functional properties.

Keywords: *Aspergillus niger,* Fermentation, Functional property, Physical property, Plant protein, Proximate composition

The global population grows continually year by year, resulting in increased seafood consumption and is expected to reach 150-160 million t by 2030 (FAO, 2020). On the other side, wild fish catches have plummeted due to overexploitation of fishery resources creating a void that can be filled by the aquaculture sector (Jannathulla *et al.,* 2019). As the aquaculture industry relies primarily on formulated feeds, its growth inevitably raises the demand for feeds supported by various raw materials. Among the ingredients, fishmeal is a predominant choice due to high protein content with balanced essential amino acids along with higher palatability and digestibility. However, increasing demand, fluctuating prices and global supply changes have underscored the importance of finding a suitable substitute to fishmeal. Though most of the research centers on plant-based proteins due to their widespread availability and inexpensive cost, the results are underwhelming due to a lack of essential amino acids and the presence of anti-nutrients. As a result, plant proteins are treated with microbial species (fermentation) in order to overcome the defects. However, most of the studies are restricted to examining only the nutrient composition after fermentation ignoring their physical properties, which is also crucial to investigate because they influence feed processing. Information on the influence of fermentation on carbohydrate sources like maize (Oladeji *et al.,* 2018), sorghum (Adiandri and Hidayah, 2019) and potato flour (Gong *et al.,* 2021), have widely been described earlier but the reports on protein sources are very scarce. The present study is therefore, aimed to examine how fermentation affects physical properties of five different plant materials that are mainly used as protein sources in aquafeed formulation.

The fungus, *Aspergillus niger* (ATCC 6275) was obtained from the Himedia Laboratories (Mumbai, India), which was cultured in potato dextrose agar (PDA) for five days at 35°C. The fungal spores were harvested using Tween 80 and were adjusted approximately to 10^7 spores ml^{-1}. Meanwhile, five different plant proteins such as soybean meal (SBM), groundnut oil cake (GNC), rapeseed meal (RSM), sunflower oil cake (SFC) and guar meal (GRM) were purchased from the local market (n=6). They were ground using an electric grinder and passed through a sieve having a mesh size of <500 µm. Deionised water was added to hydrate the ground materials resulting in final moisture content between 60 and 65%. The

hydrated mash was autoclaved at 121°C for 15 min, cooled and inoculated with 5% fungal suspension. The inoculated samples were then incubated in a BOD incubator at 35°C for three days (Jannathulla *et al.,* 2017). Post-fermentation, the fermented samples with 55% moisture were dried at 40-45°C for 48 h to have a moisture content <10%, cooled and stored in a refrigerator until further use. The proximate composition of ingredients, in terms of moisture, crude protein, crude fat, crude fiber and total ash was determined by the standard method of AOAC (1997). Nitrogen-free extract (NFE) was calculated by a difference of 100 - (Σ (%) of all the other proximate indices. Physical properties such as texture, colour and odour as well as functional properties like bulk density, water holding capacity and protein solubility index were determined according to Jannathulla *et al.* (2019). Data were subjected to two-way ANOVA to assess the effect of treatment (unfermented and fermented) and plant proteins (SBM, GNC, RSM, SFC and GRM) on functional properties. Tukey's test was used to find significant difference if any, among the categories. The difference due to fermentation between plant proteins and their counterparts was analysed using t-test.

The filamentous fungus, *A. niger* is a preferred choice for fermenting plant proteins due to its capability for producing more than twenty different hydrolytic enzymes (Pandey, 1991). The SBM and GRM had the highest crude protein content (>50%) of all the ingredients tested, while SFC had the lowest value (Table 1), regardless of the treatments. Fermentation has significantly ($p<0.05$) increased the crude protein content by 12.5%, which could be attributed to the proportionate reduction of other nutrients like crude fiber, NFE and crude fat (Rozan *et al.,* 1996). In addition, *A. niger* could bio-convert the monomers into myco-proteins such as enzymes, single cell-proteins and hydrolysed peptides by using them as a source of energy (Imelda *et al.*, 2008). Despite this, the fungus also produced certain non-protein nitrogenous substances like chitin and nucleic acids during its proliferation (Kayode and Sani, 2008), which may also be a factor contributing to the higher crude protein content in the fermented ingredients as compared to the untreated materials, in our study. Among the ingredients, the highest level of crude fat was found in GRM (7.82%) whereas it was found in the range of 0.92-2.46% in others (SBM, GNC, RSM and SFC). Fermentation significantly ($p<0.05$) reduced crude fat, which could be attributed to the production of lipase enzyme. Mala *et al.* (2007) reported that *A. niger* produced 384.3 U g^{-1} of lipase during the fermentation of agro-industrial residues. A similar reduction of crude fat was observed while fermenting SBM using *B. coagulans* (Imelda *et al.,* 2008). In contrast, a higher crude fat was reported in sesame seed meal fermented with *L. acidophilus* (Mukhopadhyay and Ray, 1999). Higashiyama *et al.* (2002) suggested that an increase of crude fat during fermentation might be attributed to the microbial fatty acid production, indicating that the lipase production varies based on the inoculum and substrate used in the fermentation process (Kamini *et al.,* 1998). Crude fiber was found to be high in SFC (27.62%) and the least value was observed in SBM and GRM (6.85 and 7.00%, respectively). Fermentation significantly ($p<0.05$) reduced crude fiber (13.44-12.44%) and NFE (31.42-26.23%), irrespective of the plant proteins. Reduction of carbohydrates, including crude fiber and NFE was

Table 1. Effect of fungal fermentation on proximate composition (% dry weight basis) of plant proteins used in feed formulation

Particulars	Proximate composition				
	Crude protein	Crude fat	Crude fiber	NFE	Total ash
Treatment					
Unfermented	45.01^{b}	3.08^{a}	13.44^{a}	31.42^{a}	7.04^{b}
Fermented	50.65^{a}	2.87^{b}	12.44^{b}	26.23^{b}	7.78^{a}
Ingredient					
SBM	56.12^{a}	0.92^{e}	6.85^{d}	28.32^{c}	7.76^{c}
GNC	47.46^{c}	2.04^{c}	12.79^{b}	29.66^{b}	8.02^{a}
RSM	44.23^{d}	2.46^{b}	10.44^{c}	35.01^{a}	7.84^{bc}
SFC	36.56^{e}	1.64^{d}	27.62^{a}	26.21^{d}	7.94^{ab}
GRM	54.76^{b}	7.82^{a}	7.00^{d}	24.92^{c}	5.47^{d}
p value					
Treatment (A)	<0.001	<0.001	<0.001	<0.001	<0.001
Ingredient (B)	<0.001	<0.001	<0.001	<0.001	<0.001
A x B	<0.001	0.007	0.001	<0.001	<0.001
SEM (+)	0.027	0.003	0.123	0.163	0.008
CV (%)	0.453	2.461	3.571	1.844	1.615

All the values are mean of three replications. Mean values bearing same superscript letters in a column within main effects and interactions between the categories do not differ significantly ($p>0.05$)

associated with the fibrolytic enzymes produced by the microorganism. Jannathulla *et al.* (2017) reported that *A. niger* was found to produce 30 U g^{-1} cellulase, 3099 U g^{-1} xylanase and 9 U g^{-1} pectinase during fermentation. The proportionate change in the nutrients might be a reason for increasing ash content in the fermented samples.

Plant-based materials, especially oilseed meals/cakes can be used as protein sources in the diet of aquatic species, including shrimp and fish. Microbial fermentation may not only influence the nutritional characteristics of plant proteins but also their physical and functional properties. The physical and functional properties of both unfermented and fermented samples are given in Table 2. Fermentation had no effect on the texture of plant proteins and all samples were homogeneous, free-flowing and dust-free. Colour of each ingredient was unique and was not greatly altered by fermentation except for SBM. After fermentation, the colour of SBM changed from somewhat yellow to brownish. Our result relating to the colour of the ingredients is in agreement with the findings of Borremans *et al.* (2020), who reported that fermented ingredients had a higher browning index and consequently deeper colour than the unfermented ones. The odour was specific, as in colour, to each ingredient tested in our study and fermented samples had slightly fermented odour along with their natural odour.

The bulk density is a measure of how much weight the samples can hold if placed directly on top of one another. The density of processed materials determines the amount and strength of packing material used, as well as the texture and mouthfeel of the product. Our results (Table 3) revealed that the bulk density of unfermented plant proteins was 0.65 g cm^{-3} and significantly ($p<0.05$) increased to 0.72 g cm^{-3} due to fermentation irrespective of the ingredients. Of all the test ingredients, the bulk density was found to be high in RSM (0.79 g cm^{-3}) and low in SBM (0.60 g cm^{-3}) regardless of the treatment. The values obtained in our study were comparable with the values reported by Okaka and Potter (1979) for cowpea (0.60 g cm^{-3}), Onimawo *et al.* (1998) for Bambara groundnut (0.60-0.75 g cm^{-3}) and Amadou *et al.* (2010) for soy protein meal. The bulk density of SBM, GNC, RSM, SFC and GRM was 0.54, 0.62, 0.79, 0.60 and 0.71 g cm^{-3}, respectively and significantly ($p<0.05$) increased by 27.1, 0.5, 0.4, 19.9 and 12.3% (Fig. 1). This result is in agreement with the fermentation of SBM using *L. plantarum* (Amadou *et al.,* 2010). The authors suggested that an increase in bulk density after fermentation might be attributed to a decrease in moisture content in the fermented samples than their counterparts. This was due to the fact that an increase in mass owing to moisture gain in the sample was lower than accompanying volumetric expansion of the bulk (Solomon and Zewdu, 2009). Similar results have been reported by Ozarslan (2002) for cottonseed and Mwithiga and Sifuna (2006) in sorghum. In contrast, Oladeji *et al.* (2018) found reduced bulk density in maize following fermentation, suggesting that this reduction could be due to macrofloral action, which breaks and utilises simple and complex carbohydrates.

Water holding capacity is defined as the amount of water absorbed by dried samples following equilibration against water vapour at a known relative humidity and it must be calculated in order to make formulation adjustments when switching protein sources. When

Table 3. Functional properties of plant proteins used in feed formulation

Particulars	Functional properties		
	BD[1] (g cm^{-3})	WHC[2] (ml g^{-1})	PSI[3] (%)
Treatment			
Unfermented	0.65[b]	3.34[a]	77.12[a]
Fermented	0.72[a]	2.99[b]	73.59[b]
Ingredient			
SBM	0.60[d]	4.29[a]	82.58[a]
GNC	0.61[d]	2.34[d]	80.05[b]
RSM	0.79[a]	2.27[d]	75.73[c]
SFC	0.66[c]	3.90[b]	64.09[d]
GRM	0.75[b]	3.03[c]	74.33[c]
p-value			
Treatment (A)	<0.001	0.001	<0.001
Ingredient (B)	<0.001	<0.001	<0.001
A x B	<0.001	0.283	0.658
SEM (+)	0.001	0.031	1.932
CV (%)	3.928	7.298	2.427

[1]Bulk density; [2]Water holding capacity; [3]Protein solubility index. All the values are mean of three replications. Values bearing same superscript letters in a column within main effects and interactions between the categories do not differ significantly ($p>0.05$)

Table 2. Effect of fungal fermentation on the physical properties of plant proteins used in feed formulation

Ingredients	Texture		Colour		Odour	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
SBM	Homogenous, free flowing and not dusty		Yellow	Brownish yellow	A slight pleasant odour of soybean	Usual odour with fermented smell
GNC			Skin colour	Skin colour	Nutty odour	
RSM			Greenish brown	Greenish brown	Natural typical aroma without any extraneous odour	
SFC			Grayish black	Grayish black	Resinous kind of smell	
GRM			Light green	Light green	Mealy odour	

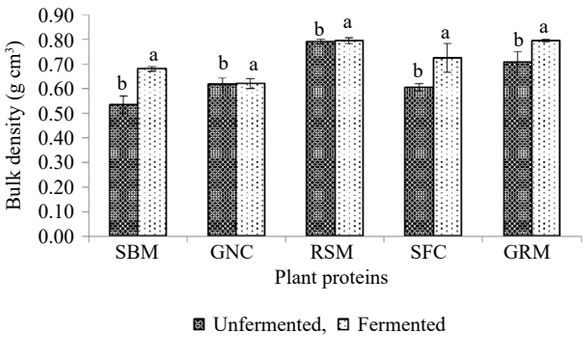

Fig. 1. Effect of fungal fermentation on bulk density of plant proteins used in aquafeed formulation. Mean values bearing same superscript letters within the categories do not differ significantly ($p>0.05$)

introduced to a formula, some proteins with a higher water retention capacity may ingest an excessive quantity of water, dehydrating other components in food systems and *vice versa*. Furthermore, water holding capacity indicates how much water is available for gelatinisation during feed preparation (Singh *et al.,* 2012). Water holding capacity was significantly ($p<0.05$) high in SBM (4.29 ml g^{-1}) followed by SFC (3.90 ml g^{-1}) and GRM (3.03 ml g^{-1}), whereas lower ($p<0.05$) values were obtained for both GNC and RSM (2.27-2.34 ml g^{-1}) regardless of the treatment. The difference in water holding capacity among the ingredients might be due to the composition and presence of carbohydrates, proteins, lipids, pH, salts and also processing methods (Kinsella and Melachouris, 1976). Fermentation significantly ($p<0.05$) decreased water holding capacity by 11.7% (Table 3) and the decrease was significantly ($p<0.05$) higher in GNC (17.7%) followed by RSM (16.4%) and SBM (13.1%). Our results corroborated with the findings of Amadou *et al.* (2010) in SBM, Obiakor-Okeke *et al.* (2014) in Mucuna seed, Okoronkwo *et al.* (2006) in Mucuna bean and Abd Elmonein *et al.* (2005) in sorghum. The authors have opined that the difference in quality of protein and reduction in the quantity of hydrophilic carbohydrates might be responsible for the variation in the water holding capacity of plant proteins after fermentation. According to Adiandri and Hidayah (2019), the higher water holding capacity of the materials was due to the amylose content, which could be used effectively by *A. niger*, resulting in lower water absorption. This could also be a reason for low water holding capacity in fermented samples in our study. However, there was no significant difference between fermented ingredients and their counterparts in SFC and GRM (Fig. 2).

Protein solubility index is an important functional attribute of protein sources as well as a good quality index that is most widely used to evaluate over processing protein sources especially SBM (Araba and Dale, 1990). The protein solubility index was 77.12% in unfermented ingredients and was significantly ($p<0.05$) reduced to 73.59% after fermentation regardless of the plant proteins. In SBM and RSM, the reduction was about 5.9 and 5.4%, respectively and in GNC and SFC, it was around 4%. Though the protein solubility index in fermented GRM was lowered by 2.6%, it did not differ significantly from its counterpart (Fig. 3). Chen *et al.* (2010) observed a similar result when fermenting SBM with *Aspergillus* alone and a combination of *Aspergillus* and *Lactobacillus*. The application of heat during autoclaving and drying processes during fermentation might have reduced protein solubility, as high protein solubility is a desired functional property and loss of solubility is commonly used as an indicator of protein denaturation. Plant protein sources must not be exposed to excessive heat to maintain optimal nutritional value, as this will denature the protein pool, making it less soluble and digestible. Despite the fact that heat treatment reduced the protein solubility index, Parsons *et al.* (1991) and Jannathulla *et al.* (2017) found no impairment in growth in terms of weight or feed efficiency in chick, pig and shrimp with protein solubility in KOH more than 72, 59 and 66%, respectively. Akkerman (2014) suggested that heat treatment denatured the protein by altering structural integrity, particularly secondary and tertiary structure, as well as weakening hydrogen bonds and functional groups. It twists, spins and bends proteins, converting them from folded to unfolded states, resulting in the loss of functional activity. However, the protein solubility in all of the fermented samples in our investigation was greater than 73-80% (Fig. 3). Percentage variation in functional properties was comparatively high for bulk density and low for water holding capacity and protein solubility index (Fig. 4). In conclusion, the fungus, *A. niger* did not only influence the chemical composition but also the physical and functional properties of the plant proteins, suggesting that fungal fermentation could enhance the utility of plant proteins by improving the processing characteristics.

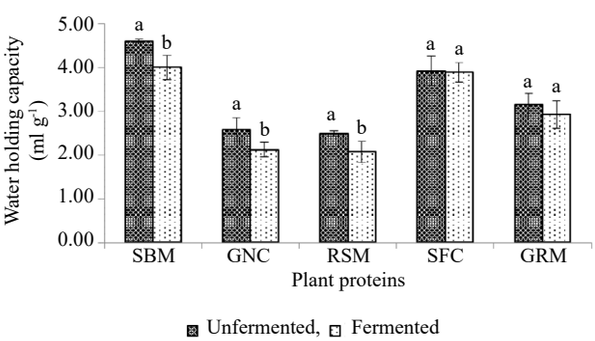

Fig. 2. Effect of fungal fermentation on water holding capacity of plant proteins used in aquafeed formulation. Mean values bearing same superscript letters within the categories do not differ significantly ($p>0.05$)

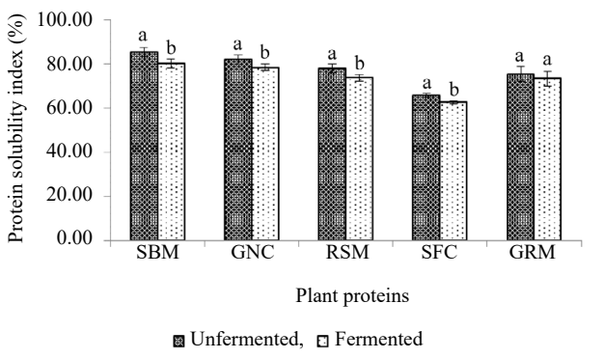

Fig. 3. Effect of fungal fermentation on protein solubility index of plant proteins used in aquafeed formulation. Mean values bearing same superscript letters within the categories do not differ significantly (p>0.05)

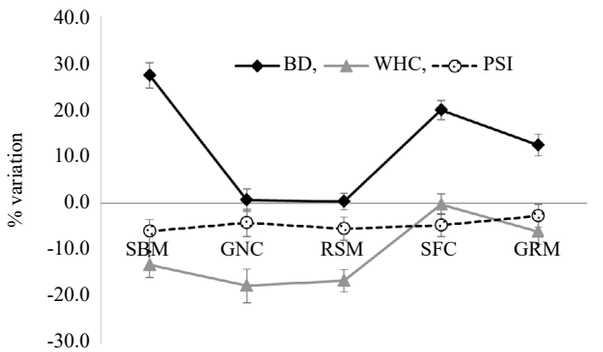

Fig. 4. Percentage variation in functional properties between unfermented and fermented plant proteins used in feed formulation

Acknowledgements

The authors are grateful to the Director, ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India for the support extended to carry out the study. Dr. J. Syama Dayal, acknowledges the DBT Project: BT/PR32691/AAQ/3/957/2019 for financial support.

References

Abd Elmoneim, O. E., Schiffler, B. and Bernhardt, R. 2005. Effect of fermentation on the functional properties of sorghum flour. *Food Chem.,* 92: 1-5. https://doi.org/10.1016/j.foodchem.2004.05.058.

Adiandri, R. S. and Hidayah, N. 2019. Effect of fermentation using *Lactobacillus casei* on the physicochemical and functional properties of sorghum flour. *IOP Conference Series: Earth and Environmental Science.* IOP Publishing, p. 012-025. https://doi.org/10.1088/1755-1315/309/1/012025.

Akkerman, M. 2014. *The effect of heating process on milk whey protein denaturation and rennet coagulation properties.* Dissertation, Aarhus university, Denmark.

Amadou, I., Amza, T., Foh, M. B. K., Kamara, M. T. and Le, G. W. 2010. Influence of *Lactobacillus plantarum* Lp6 fermentation on the functional properties of soybean protein meal. *Em. J. Food Agric.,* 22(6): 456-465. https://doi.org/10.9755/ejfa.v22i6.

AOAC 1997. *Official methods of analysis*, 18th edn. Association of Official Analytical Chemists, Benjamin Franklin Station, Washington DC, USA.

Araba, M. and Dale, N. M. 1990. Evaluation of protein solubility as an indicator of under processing of soybean meal. *Poult. Sci.,* 69: 1749-1752. https://doi.org/10.3382/ps.0691749.

Borremans, A., Smets, R. and Van Campenhout, L. 2020. Fermentation *versus* meat preservatives to extend the shelf-life of mealworm (*Tenebrio molitor*) paste for feed and food applications. *Front. Microbiol.,* 11: 1510. https://doi.org/10.3389/fmicb.2020.01510.

Chen, C. C., Shih, Y. C., Chiou, P. W. S. and Yu, B. 2010. Evaluating nutritional quality of single stage and two stage fermented soybean meal. *Asian-Australasian J. Anim. Sci.,* 23: 598-606. https://doi.org/10.5713/ajas.2010.90341.

FAO 2020. *Summary tables of fishery statistics: Capture aquaculture, commodity and food balance sheets.* Fisheries and Aquaculture Department, Food and Agriculture Organisation of the United Nations, Rome, Italy. http://www.fao.org/fishery/statistics/en, 2003.

Gong, S., Yu, Y., Li, W., Wu, J. and Wang, Z. 2021. Effects of amylolytic, *Lactobacillus* fermentation on the nutritional quality and digestibility of purple potato flour. *J. Food Comp. Anal.*, 104363. https://doi.org/10.1016/j.jfca.2021.104363.

Higashiyama, K., Fujikawa, S., Park, E. Y. and Shimizu, S. 2002. Production of arachidonic acid by *Mortierella* fungi. *Biotechnol. Biopro. Eng.,* 7: 252-262. https://doi,org/10.1007/BF02932833.

Imelda, J., Paulraj, R. and Bhatnagar, D. 2008. Effect of solid-state fermentation on nutrient composition of selected feed ingredients. *Indian J. Fish.,* 55: 327-332.

Jannathulla, R., Ambasankar, K., Syama Dayal, J., Rajamohmed, M. K., Sravanthi, O. and Rajesh, B. 2019. Studies on functional properties and chemical composition of commercial shrimp feeds used in Andhra Pradesh, India. *Int. J. Fish. Aqua. Stud.,* 7: 84-89.

Jannathulla, R., Dayal, J. S., Ambasankar, K., Khan, H. I., Madhubabu, E. P. and Muralidhar, M. 2017. Effect of protein solubility of soybean meal on growth, digestibility and nutrient utilisation in *Penaeus vannamei*. *Aquac. Int.,* 25: 1693-1706. https://doi.org/10.1007/s10499-017-0147-9.

Jannathulla, R., Dayal, J. S., Vasanthakumar, D., Ambasankar, K. and Muralidhar, M. 2017. Effect of fermentation methods on amino acids, fiber fractions and anti-nutritional factors in different plant protein sources and essential amino acid index for *Penaeus vannamei* Boone, 1931. *Indian J. Fish.,* 64: 40-47. https://doi.org/10.21077/ijf.2017.64.2.60341-07.

Jannathulla, R., Rajaram, V., Kalanjiam, R., Ambasankar, K., Muralidhar, M. and Dayal, J. S. 2019. Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilisation of plant protein sources. *Aquac. Res.,* 50: 3493-3506. https://doi.org/10.1111/are.14324.

Kamini, N. R., Mala, J. G. S. and Puvanakrishnan, R. 1998. Lipase production from *Aspergillus niger* by solid-state fermentation using gingelly oil cake. *Proc. Biochem.,* 33: 505-511. https://doi.org/10.1016/S0032-9592(98)00005-3.

Kayode, R. M. O. and Sani, A. 2008. Physicochemical and proximate composition of mango (*Mangifera indica*) kernel cake fermented with mono-culture of fungal isolates obtained from naturally decomposed mango kernel. *Life Sci. J.,* 5: 55-63.

Kinsella, J. E. and Melachouris, N. 1976. Functional properties of proteins in foods: A survey. *Cr. Rev. Food Sci. Nutr.,* 7: 219-280. https://doi.org/10.1080/10408397609527208.

Mala, J. G. S., Edwinoliver, N. G., Kamini, N. R. and Puvanakrishnan, R. 2007. Mixed substrate solid state fermentation for production and extraction of lipase from *Aspergillus niger* MTCC 2594. *J. Gen. Appl. Microbiol.,* 53: 247-253. https://doi.org/10.2323/jgam.53.247.

Mukhopadhyay, N. and Ray, A. K. 1999. Improvement of quality of sesame *Seasamum indicum* seed meal protein with supplemental amino acids in feeds for rohu *Labeo rohita* (Hamilton) fingerlings. *Aquac. Res.,* 30: 549-557. https://doi.org/10.1046/j.1365-2109.1999.00346.x.

Mwithiga, G. and Sifuna, M. M. 2006. Effect of moisture content on the physical properties of three varieties of sorghum seeds. *J. Food Eng.,* 75: 480-486. https://doi.org/10.1016/j.jfoodeng.2005.04.053.

Obiakor-Okeke, P. N., Obioha, B. C. and Onyeneke, E. N. 2014. Nutrient and sensory evaluation of traditional soups consumed in Igbere community in Bende local government area, Abia State, Nigeria. *Int. J. Nutr. Food Sci.,* 3(5): 370-379.

Okaka, J. C. and Potter, N. N. 1979. Physico-chemical and functional properties of cowpea powders processed to reduce beany flavor. *J. Food Sci.*, 44: 1235-1240. https://doi.org/10.1111/j.1365-2621.1979.tb03488.x.

Okoronkwo, C. U., Agoha, E. E. C., Ogodo, A. C. and Nwachukwu, N. O. 2006. Nutritional and physical properties of weaning food flour based on maize and African yam bean seeds. *Int. J. Adv. Eng. Manag.,* 1: 34-37.

Oladeji, B. S., Irinkoyenikan, O. A., Akanbi, C. T. and Gbadamosi, S. O. 2018. Effect of fermentation on the physicochemical properties, pasting profile and sensory scores of normal endosperm maize and quality protein maize flours. *Int. Food Res. J.,* 25: 1100-1108.

Onimawo, I. A., Momoh, A. H. and Usman, A. 1998. Proximate composition and functional properties of four cultivars of bambara groundnut (*Voandezeia subterranea*). *Plant Foods Human Nutr.,* 53: 153-158. https://doi.org/10.1023/A:1008027030095.

Ozarslan, C. 2002. PH - Postharvest technology: Physical properties of cotton seed. *Biosys. Eng.,* 2: 169-174. https://doi.or/10.1006/bioe.2002.0105.

Pandey, A. 1991. Effect of particle size of substrate of enzyme production in solid-state fermentation. *Biores. Technol.,* 37: 169-172. https://doi.org/10.1016/0960-8524(91)90206-Y.

Parsons, C. M., Hashimoto, K., Wedekind, K. J. and Baker, D. H. 1991. Soybean protein solubility in potassium hydroxide: An *in vitro* test of *in vivo* protein quality. *J. Anim. Sci.,* 69: 2918-2924. https://doi.org/10.2527/1991.6972918x.

Rozan, P., Villaum, C., Bau, H. M., Schwertz, A., Nicolas, J. P. and Mejean, L. 1996. Detoxication of rapeseed meal by *Rhizopus oligosporus* sp-T3: A first step towards rapeseed protein concentrate. *Int. J. Food Sci. Technol.,* 31: 85-90. https://doi.org/10.1111/j.1365-2621.1996.17-315.x.

Singh, K., Pandey, V. C., Singh, B. and Singh, R. R. 2012. Ecological restoration of degraded sodic lands through afforestation and cropping. *Ecol. Eng.,* 43: 70-80. https://doi.org/10.1016/j.ecoleng.2012.02.029.

Solomon, W. K. and Zewdu, A. D. 2009. Moisture-dependent physical properties of *niger* (*Guizotia abyssinica* Cass.) seed. *Ind. Crops Products*, 29(1): 165-170. https://doi.org/10.1016/j.indcrop.2008.04.018.

Date of Receipt : 31.01.2022
Date of Acceptance : 23.06.2022