ICBN-04

INTERNATIONAL CONFERENCE ON BIOTECHNOLOGY AND NEUROSCIENCE

DECEMBER 29-31, 2004

VENUE :
POLYMER SCIENCE AUDITORIUM, CUSAT
ELECTRONICS AUDITORIUM, CUSAT
MUNICIPAL TOWN HALL, KALAMASSERY

ABSTRACTS & PROCEEDINGS

Organised by

CENTRE FOR NEUROSCIENCE
DEPARTMENT OF BIOTECHNOLOGY
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

SOCIETY FOR BIOTECHNOLOGISTS (INDIA)

SOCIETY FOR NEUROCHEMISTRY (INDIA)

CHANGES IN NUTRIENT PROFILE OF WHEAT BRAN BY SOLID-STATE FERMENTATION USING *ASPERGILLUS NIGER*

Imelda-Joseph, Bhatnagar, D., Paulraj, R. and Shylaja, G
Central Marine Fisheries Research Institute, Post Box No. 1603
Ernakulam North P.O., Cochin- 682 018, Kerala, India.

Abstract

Aquaculture production has expanded at a rate of 15% per year and is predicted to continue at this rate for at least the next decade. Demand on traditional feed ingredients like fishmeal and fish oil is increasing and expanded production of carnivorous species requiring high protein, high energy feeds will further tax global fishmeal and oil supplies. Grain and oil seed by products are the most likely candidate feed sources to carry aquaculture forward to higher production levels. Wheat bran, the main by product of wheat milling is a heterogeneous mixture of grain fragments containing hyaline aleurone layers of the seed. The high quality proteins, minerals and vitamins originally located in the outer layers of the kernel are concentrated in the bran, which therefore becomes a very rich source of nutrients. However, the nutritive value of bran, as of any other product, is not only a result of its total nutrient content but also on nutrient availability and digestibility. Solid-state fermentation (SSF) is defined as the fermentation of solid substrates in the absence of free flowing water. Recently SSF has gained interest due to its potential in solid waste treatment, the production of secondary metabolites and production of novel foods and feed ingredients. Present investigation was concerned with the solid-state cultivation of the fungus *Aspergillus niger* isolated from mangrove ecosystem on wheat bran and its impact on its nutritional value. The crude protein content increased significantly ($P<0.05$) with the maximum increase on day eight (102.4%). Significant ($P<0.05$) reduction in total carbohydrate with least on day 8 was observed (21.64% reduction). Variation in amino acid profile was also observed during fermentation. Duration of fermentation had significant ($P<0.05$) effect on the nutritional profile of the substrate. The results of the present study show that the present strain of *A. niger* can be effectively used for bioconversion of wheat bran for use in aquafeed formulations.

Introduction

aquaculture to make a net contribution to human food supplies, the present use of fishmeal in aquafeeds must be substantially reduced (Williams *et al.*, 2003). Alternative feed sources are increasingly being sought to provide a cost effective means to supply cultured fish and crustacean species with the same nutrients offered by fishmeal and fish oils enabling efficient physiological function, reproduction and commercially viable growth rates (Jobling *et al.*, 2001). When plant meals are used as fishmeal supplements, anti nutritional factors such as phytic acid, alkaloids, tannins, and protease inhibitors adversely affect the efficient partitioning of proteins (Singh *et al.*, 2003). Also plant meals are limited by containing insufficient levels of essential amino acids such as cysteine, methionine and lysine (Ali, 1992). Wheat and wheat products are widely in aquafeeds for improving pellet stability and it is suggested to use wheat bran instead for cost effectiveness (Maina *et al.*, 2002). Wheat bran, the main by product of wheat milling, contains high quality proteins, minerals and vitamins. But it is less digestible and the complex matrix of the cell walls acts as a barrier to digestive enzymes. Moreover, anti nutrients like, phytic acid limit the availability of bran nutrients (Lena *et al.*, 1997). Mangrove ecosystem harbors a wide variety of microorganisms like, bacteria, fungi and micro algae (Kathiresan and Bhingam, 2001). Filamentous fungi are used for various industrial fermentation processes for food and metabolite production (Pandey *et al.*, 1999). *Aspergillus niger* is reported to produce as many as 19 enzymes in solid state fermentations (Pandey *et al.*, 1999). Fermentation with *Aspergillus niger*, have been studied for citric acid, xylanolytic enzyme, pectolytic enzyme, d-glucosidase, alpha and gluco-amylase, and nucleic acid related substances productions (Tello-Solis*et al.*, 1994; Pandey *et al.*, 1999; Bhatnagar, 2004). Solid- state fermentation (SSF) is the cultivation of microorganisms on selected substrate in the absence of free water. The study of fungal growth in SSF shows advantages over submerged cultures, because this is a natural environment for filamentous fungi. SSF for feed production include improvement in the digestibility, nutrient bioavailability and protein value of feedstuffs (Gumbira-Said, 1996). Depending upon the kind and extent of treatment, the substrates get upgraded in protein (3.5 times increase in fungal protein content) fats, soluble sugars, vitamins, amino acids and thus can even be used in entirety as animal feed (Singh *et al.*, 1990; Puniya and Singh, 1995; Mitra *et al.*, 1996). SSF can add aroma, palatability and health promoting substances of interest to the substrate. Several fungal species have been

used for SSF in several countries, especially in Asia for preparing fermented foodstuffs such as tempeh (Zheng and Shetty, 1998). Fermentation of cassava with *Aspergillus, Neurospora* and *Rhizopus* elevated the protein values on using nitrogenous supplements (Varghese *et al.*, 1976; Balagopalan and Padmaja, 1988; Stertz *et al.*, 1999). The SSF of soybean flour using *Bacillus coagulans* improved the protein and NFE contents of the substrate, along with reduction in crude fibre (Imelda-Joseph and Paulraj, 2003). SSF of oilcakes using *Aspergillus niger* Strain 616 and *Bacillus coagulans* resulted in enrichment of protein, and the fermented product was effective as fishmeal replacement (20%) in shrimp diets (Vijayakumar, 2003).

Materials and Methods

Wheat bran for fermentation was bought from an animal feed shop at Cochin. Flasks (500 ml) with 20 g of wheat bran fortified with Czapek Dox [$NaNO_3$ (2.5 g l^{-1}), K_2HPO_4 (1 g l^{-1}), $MgSO_4$. $7H_2O$ (0.5 g l^{-1}), KCl (0.5 g l^{-1}) pH @5.0] were used for the study (Aikat and Bhattacharya, 2000). Moisture content was adjusted to 60% by addition of Czapek- Dox. Before fermentation, flasks with the moist substrate were autoclaved at 121.1 ^{0}C for 15 min.

For inoculum, 7 days old slants of *A. niger* strain S_14 maintained in potato dextrose agar (PDA) were taken and 10 ml of sterile Tween-80 (0.1%) was added to make a spore suspension. An inoculum size of 0.5 ml containing $20x10^6$ spores was used for each flask and incubated at 30^{0}C ±1 (Kaur *et al.*, 2003). All flasks were kept under stationary condition, with occasional shaking at pH 6.4 - 6.5 for optimization of duration. Three replicates of each treatment were kept for 8 days, with sampling after every 24 h interval starting from day 0 to day 8 (Day, 0,1,2,3,4,5,6,7 and 8). After drying to a constant weight at 85°C, composition of fermented wheat bran (dry matter, crude protein, crude ash, crude fiber, and crude fat) was estimated by following AOAC (1990). Amino acid profile was determined using HPLC (Waters India Ltd.). The results were analyzed by two-way ANOVA.

Results and Discussion

In the present study, wheat bran, a major agro industrial by-product was used for solid-state fermentation. It has been widely used as a supplement in cattle feed (Mitra *et al.*, 1996; Lena *et al.*, 1997; Aikat and Bhattacharya, 2000; Kaur *et al.*, 2003). The

ochemical composition of control and the fermented wheat bran were analyzed and the sults are given in Table 1. Crude protein content in the control was 13.66%, which then gradually increased with duration of fermentation and was found to be 16.86% on day one and 27.6% on day 8. An increase above 102.4% was obtained on day 8 (Table 2). This significant ($P<0.05$) increase in protein content during fermentation may be tributed to the efficient bioconversion of highly polymerized carbohydrates into fungal otein and the production of different types of enzymes, which are proteinaceous in ature (Vijayakumar, 2003). Lena *et al.* (1997) have also reported increase in crude protein content of wheat bran during SSF with white-rot fungus. Significant increase in the crude protein and true protein contents, protein solubility and *in vitro* digestibility of chickpea by SSF have been reported with increase in the amino acid and fatty acid ontents especially the available lysine, palmitic acid and stearic acid (Moreno *et al.*, 000). Mitra *et al.* (1996) have reported that by the process of SSF it was possible to convert cassava to a protein enriched animal feed and the highest increase in protein content observed was 14.32% from the initial 1.28% by filamentous fungi.

The amino acid profile of control as well as the fermented wheat bran is shown in Table 3. After 5 days (120 h) of fermentation the protein increased by 77.16%. Significant increase in production of Aspartic acid (43.71%), serine (69.79%), histidine (6.93%), threonine (70.57%), alanine (36.69%), valine (16.8%), cysteine (40%) and lysine (43.77%) were observed in 5 days. The level of amino acids in cellobiases, the enzymes produced by certain strains of *A.niger* showed high contents of aspartic acid, glutamic acid, threonine, serine, and glycine (Abdel-Naby *et al.*, 1999). Significant increase in crude protein and true protein contents, protein solubility and *in vitro* digestibility of chickpea by SSF have been reported with increase in the amino acid and fatty acid contents especially the available lysine, palmitic acid and stearic acid (Moreno *et al.*, 2000). Single cell protein produced by *A. niger* contained 30.4% crude protein and had an essential amino acid profile featuring a high lysine content and appreciable amounts of methionine and tryptophane, and 12.9% fat, which was comprised of all essential fatty acids (Singh *et al.*, 1991). The increase in amino acids in the fermented product shows that the carbohydrate consumption is closely proportional to protein production during solid substrate fermentation. Reduction in Glutamic acid (43.94%), glycine (5.8%), Proline (47.19%), methionine (67.64%) and tryptophan (34.01%) were also observed. This may be due to the utilization of these amino acids for the production

of enzymes and other organic compounds by *A. niger* during SSF. The gradual increase in crude fat content in the fermented wheat bran during SSF till day 8 may be attributed to the production fungal fatty acids during fermentation. Fungi are reported to produce fatty acids at varying levels during SSF (Higashiyama *et al.*, 2002).

The crude ash content of the control was 4.69%, but afterwards it increased up to 6.62% on day 4 and thereafter gradually reduced. Total carbohydrate content was determined by indirect estimation by subtracting the total values of crude fat, crude protein and crude ash from 100. In control the total carbohydrate was 80.46%. Further it reduced with duration of fermentation. The lowest value was obtained on day 8 (63.05%). The total carbohydrate loss was significant ($P<0.05$) with duration of fermentation. A total loss of 63.05% was observed on day 8 (Table 2). The total carbohydrate content showed a steady and significant ($P<0.05$) decrease during fermentation possibly due to the breakdown of carbohydrate by the action of fungal amylases, releasing the simple and utilizable carbohydrate molecules for its metabolic activities. The reduction of total carbohydrates from 80.46% in control to 63.05% (*i.e.* © 21.64% reduction) on day 8 in the fermentation process shows the continuous utilization of carbohydrates for the metabolic activities of *A. niger*. The results of the present study suggest that the selected *A. niger* strain $S_1 4$ is an efficient one to convert complex carbohydrates to simpler molecules with enrichment of fungal protein.

Table 1 Composition of fermented wheat bran (on dry matter basis)

Days	Crude protein	Crude Ash	Crude Fat	Total CHO**
0 (control)	13.66±1.15	4.69±0.07	1.19±0.02	80.46 ±1.2
1	16.86±0.09	4.42±0.03	2.49±0.06	76.24±0.1
2	19.77±0.51	4.42±0.03	3.11±0.04	72.71±0.58
3	20.89±0.02	5.23±0.19	3.7±0.07	70.2±0.27
4	22.86±0.03	6.62±0.29	4.27±0.05	66.26±0.28
5	24.22±0.5	5.81±0.23	4.47±0.04	65.51±0.39
6	25.22±0.32	5.28±0.24	4.68±0.04	65.11±0.05
7	26.24±0.15	5.17±0.1	4.66±0.08	63.94±0.18

8	27.6±0.25	4.56±0.19	4.8±0.01	63.05±0.08

*Acid Insoluble Ash; ** Total Carbohydrate

Table 2 Variation in percentage carbohydrate and protein of wheat bran during SSF using $2x10^6$ spores

Days	Dry matter (%)	Carbohydrate (%)	CHO* Reduction (%)	Crude protein (%)	Protein Increase (%)
0 (control)	97.65±0.08	80.46±1.2	----	13.66±1.15	----
1	97.60±0.14	76.24±0.1	5.24	16.86±0.09	23.42
2	97.69±0.17	72.71±0.58	9.63	19.77±0.51	44.73
3	97.9±0.13	70.2±0.27	12.75	20.89±0.02	52.93
4	97.77±0.18	66.26±0.28	17.6	22.86±0.03	67.35
5	96.97±0.32	65.51±0.39	18.58	24.22±0.5	77.16
6	97.13±0.21	65.11±0.05	19.07	25.22±0.32	84.63
7	96.69±0.46	63.94±0.18	20.53	26.24±0.15	92.09
8	97.4±0.01	63.05±0.08	21.64	27.6±0.25	102.04

*Carbohydrate

Table 3 The amino acid percentage in the control and fermented wheat bran over 8 days

Amino Acid	Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
ASP	**7.07**	7.1	8.01	9.26	9.29	**10.16**	10.09	8.89	8.85
GLU	**16.84**	17.37	15.27	10.45	12.26	**9.44**	9.44	9.5	10.28
SER	**5.43**	5.93	7.04	7.64	8.23	**9.22**	9.07	7.98	7.25
GLY	**11.31**	11.3	11.29	11.88	11.26	**10.65**	10.82	11.29	11

HIST	**2.31**	2.45	2.58	2.41	2.41	**2.47**	2.46	2.29	2.5
ARG	**4.34**	4.33	4.02	3.98	3.89	**3.69**	**3.69**	3.22	3.75
THR	**3.5**	3.9	4.55	5.21	5.33	**5.97**	5.86	6.4	5.32
ALA	**7.25**	7.37	7.76	9.27	8.74	9.17	9.16	**9.91**	9.1
PRO	**11.76**	10.38	9.81	7.22	7.49	**6.21**	6.05	7.08	7.09
TYR	2.43	2.39	2.39	2.66	2.49	2.44	2.41	2.41	2.61
VAL	**4.94**	5.38	5.69	6.21	5.94	**5.77**	5.82	5.67	5.99
MET	**0.34**	0.18	0.11	0.11	0.1	0.12	**0.11**	0.11	0.05
CYS	**0.15**	0.21	0.24	0.21	0.2	0.21	**0.24**	0.14	0.12
ISOL	**3.23**	3.4	3.67	4.24	3.93	3.8	**3.92**	3.91	3.86
LEU	7.41	7.34	7.05	7.76	7.44	7.22	7.42	7.87	7.52
PHE	**3.99**	3.85	3.53	3.66	3.51	3.23	3.38	3.54	3.42
LYS	**5.14**	4.55	3.93	6.25	5.62	7.2	**7.39**	6.73	8.46
TRP	**2.94**	2.25	1.83	2.16	1.14	1.75	**1.94**	1.6	1.78

Conclusion

The present study shows that the *Aspergillus niger* strain $S_1 4$, which has been isolated from the mangrove swamp has considerably improved the nutrient profile of the wheat bran during SSF and it can be effectively used for bioconversion of cheaper agricultural by- products to be used in aquafeed as minor or major ingredient based on the nutritional requirement of the cultured organism.

Acknowledgements

The authors are grateful to Dr. Mohan Joseph Modayil, Director, CMFRI, Cochin for the facilities provided to carry out this work. The technical assistance rendered by

Shri. S. Nandakumar Rao, Technical Assistant, Physiology Nutrition and Pathology Division, CMFRI, Cochin is gratefully acknowledged.

References

Abdel-Naby MA, Osman MY, Abdel-Fattah AF., 1999. Purification and properties of three cellobiases from Aspergillus niger A20. *Appl Biochem Biotechnol.* 76(1):33-44

Aikat, K., and Bhattacharya, B.C., 2000. Protease extraction in solid state fermentation of wheat bran by a local strain of *Rhizopus oryzae* and growth studies by the soft gel technique. *Proc. Biochem.*, 35: 907-914.

Ali, S.A., 1992. Evaluation of some animal and plant protein sources in the diet of the shrimp *Penaeus indicus. Asian Fish. Sci.*,5: 277- 289.

AOAC, 1990. Official methods of Analysis of the Association of Analytical Chemists, 15th edn., Association of Analytical Chemists, Inc., Arlington, USA, 1298p.

Balagopalan, C. and Padmaja, G., 1988. Protein enrichment of cassava flour by solid-state fermentation with *Trichoderma pseudokoninghi* Rifai for cattle feed, *In* Proc. International Society for Tropical Root Crops, Bangkok, pp. 426-432.

Bhatnagar, B., 2004. Amylase and protease production by solid-state fermentation using *Aspergillus niger* from mangrove swamp, M.F.Sc. (Mariculture) Dissertation, Submitted to Central Institute of fisheries Education, Mumbai, India, 63p.

Gumbira-Said, E., 1996. Packed bed solid-state cultivation systems for the production of animal feed. *J. Sci. Ind. Res.*, 55: 431-438.

Higashiyama, K., Fujikawa, S., Park, E.Y., and Shimizu, S., 2002. Production of arachidonic acid by *Mortierella* fungi. *Biotechnol. Bioprocess Eng.*, 7: 252- 262.

Imelda-Joseph and Paulraj, R. 2003. Fermented soybean flour as a fishmeal substitute in diets of juvenile tiger shrimp, *Penaeus monodon. In:* National Conference on Aquaculture Nutrition, NATP&CMFRI, Kochi, Abst. 20: 51-53.

Jobling, M., Gomes, E., Dias, J., 2001. Feed types, manufacture and ingredients. *In:*Houlihan, D., Boujard, T., Jobling, M., (eds). Food Intake in Fish. Pp. 25-48. Iowa state University Press, IA, USA.

Kathiresan, K. and Bingham, B.L., 2001. Biology of mangroves and mangrove ecosystems. *Adv. Mar. Biol.*, 40: 81-251.

Kaur, P.K., Grewal, H.S. and Kocher, G.S. 2003. Production of α-amylase by *Aspergillus niger* using wheat bran in submerged and solid state fermentations. *Indian J. Microbiol.*, 43(2): 143-145.

Lena D. G., Patroni, E. and Quaglia, G. B., 1997. Improving the nutritional value of wheat bran by a white rot fungus. *International J. Food Sci. Technol.*, 32: 513-519.

Mairia, J.G., Beames, R.M., Higgs, D., Mbugua, P.N., Iwama, G., Kisia, S.M., 2002. Digestibility and feeding value of some feed ingredients fed to tilapia *Oreochromis niloticus* (L.). *Aquaculture Research*, 33: 853-862.

Mitra. P., Chakraborty. R. and Chandra, A. L., 1996. Production of proteolytic enzymes by solid-state fermentation. *J. Sci. Ind. Res.*, 55: 439-442.

Pandey, A., Selvakumar, P., Soccol, C.R. and Nigam, P. 1999. Solid state fermentation for the production of industrial enzymes. *Curr. Sci.*, 77(1): 149-162.

Puniya, A. K. and Singh, K., 1995. Biochemical changes during the solid substrate fermentation of wheat straw. *Indian. J. Microbiol.*, 35(3): 211-215.

Tello-Solis, S.R., Rodriguez-Romero, A., Hernandez-Arana, A., 1994. Circular dichroism studies of acid proteinases from *Aspergillus niger* and *Aspergillus awamori* Biochem Mol. Biol Int., 33(4): 759-68.

Singh, A., Abidi A.B., Agrawal A.K., Darmwal N.S., 1991. Single cell protein production by Aspergillus niger and its evaluation. *Zentralbl Mikrobiol.*, 146(3):181-4.

Singh, K., Linden, C. J., Johnson, E. J. and Tengerdy, P. R., 1990. Bioconversion of wheat straw to animal feed by solid substrate fermentation or ensiling. *Indian J. Microbiol.*, 30(2):201-208.

Singh, K., Garg, S.K., Kallla, A. and Bhatnagar, 2003. Oilcakes as protein sources in supplementary diets for the growth of *Cirrhinus mrigala* (Ham.) fingerlings: laboratory and field studies. *Bioresource Technology*, 86: 283- 291.

Sterz, S. V., Shough, S. T. and Walter, K., 1999. Solid-state fermentation of raw cassava by *Rhizopus formosa* for improving the nutritional quality. *J. Appl. Microbiol. Biot.*, 18:57-62.

Varghese, G., Thambiraj, J.J. and Wong, F. M., 1976. Protein enrichment of cassava by fermentation with microfungi and the role of natural nitrogenous supplements. Proceedings of the fourth international Symposium of the International Society of root Crops (ed. Cock J Macintyre R and Graham, M), CIAT, Cali, Columbia, 1-7 August 1976, IDRC, Ottawa 080e, pp. 250-255.

Vijayakumar, M., 2003. Solid state fermentation of oil cakes and wheat flour and evaluation of the products in shrimp feed, M.F.Sc. (Mariculture) Dissertation, Submitted to Central Institute of fisheries Education, Mumbai, India, 85p.

Williams, K.C., Barlow, C.g., Rodgers, L., and Ruscoe, I., 2003. Potential of meat meal to replace fishmeal in extruded dry diets for barramundi, *Lates calcarifer* (Bloch). I.Growth performance. *Aquaculture Research*, 34: 23-32.

Zheng, Z. and Shetty, K., 1998. Solid-state production of beneficial fungi on apple processing wastes using glucosamine as the indicator of growth. *J. Agric. Food. Chem.*, 46:783-787.