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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

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ABSTRACT

The incorporation of plant protein sources in shrimp feed is limited due to unbalanced amino acids and higher anti-nutrients. In the present study, soybean meal (SBM), groundnut oil cake (GNC), rapeseed meal (RSM), sunflower oil cake (SFC) and guar meal (GRM) were subjected to natural, bacterial, fungal and yeast fermentation methods. The essential amino acid contents were increased by 4-28% in SBM, 7-26% in GNC, 3-27% in RSM, 8-18% in SFC and 4-14% in GRM. The increase was better for lysine with fungal fermentation (2.31-4.01%). The improvement in other limiting amino acids *viz*., methionine and tryptophan also showed positive response to fermentation. The analytical results showed improved essential amino acid index (EAAI) in the fermented ingredients and the increase was better with RSM (0.82 to 0.92) using *Aspergillus niger*. Fiber fractions were reduced ($p<0.05$) in fungal and yeast treated samples but not due to natural or bacterial fermentation. The reduction of cellulose and hemicellulose was not only influenced by the inoculum but also on the ingredient used. The reduction of anti-nutrients ($p<0.05$) such as trypsin inhibitor, phytic acid, saponin, tannin, glucosinolate and guar gum were found to be lower in natural fermentation than other methods. The results indicated that fungal fermentation is more suitable for improving the nutritional quality of plant protein sources and this data will pave way for higher fishmeal replacement in shrimp feed formulations.

Keywords: Anti-nutrients, Essential amino acid index, Microorganisms, Plant protein sources, Solid state fermentation

Introduction

Fishmeal is the most common and expensive protein source used in aquafeed formulations. Commercially, it is included at above 20% in shrimp feed formulation since being highly digestible, palatable and also having excellent amino acid profiles and other essential nutrients to fulfill the dietary requirement of cultured species. Commercial shrimp feed production is expected to increase from 0.9 million t in 1995 to 9.2 million t in 2020 (Tacon and Metian, 2008). The sustainability of aquaculture growth depends on the ability of the nutritionists to find alternatives to fishmeal as the demand exceeds to its supply by 2050 (Halweil, 2008). Plant protein sources, especially certain oil cakes might be considered as a viable alternative to an extent due to their sustainable availability and reasonable price. The use of high levels of plant protein sources resulted in reduction in digestibility and growth performance in shrimp (Dayal *et al.,* 2011). The lower performance of the plant protein sources was attributed to the deficiencies in essential amino acids like tryptophan, lysine and sulphur containing amino acids, higher content of fiber fractions and anti-nutrients by affecting digestibility and palatability of feed (Akiyama, 1991).

Solid state fermentation is reported as a viable processing technique to reduce the undesired substances and to enrich the nutritional quality of the agricultural residues (Shi *et al.,* 2015). However, the information on effect of inoculums used for fermentation on fiber fractions and the overall essential amino acid index (EAAI) of plant protein sources for shrimp is not available. Hence, in the present study, five most potential plant protein sources *viz*., soybean meal (SBM), groundnut oil cake (GNC), rapeseed meal (RSM), sunflower oil cake (SFC) and guar meal (GRM) were treated by four different ways of solid state fermentation (SSF) (natural, bacterial, fungal and yeast) to know the suitability of the inoculums. The effect of fermentation was evaluated based on the essential amino acid requirements of Pacific whitelegged shrimp, *Penaeus vannamei* Boone, 1931 by calculating EAAI along with the reduction (%) of both fiber fractions and anti-nutrients. The present results provide the baseline

data to maximise the utilisation of these ingredients in shrimp feed formulations with simultaneous reduction of pressure on fishmeal demand.

Materials and methods

Ingredients and fermentation methods

Commercial feed ingredients such as SBM, GNC, RSM, SFC and GRM were purchased. The coarse ingredients were ground to fine particles lesser than 500 µm and stored.

The microorganisms *Lactobacillus acidophilus* (ATCC 4356), *Aspergillus niger* (ATCC 6275) and *Saccharomyces cerevisiae* (ATCC 9763) obtained from Himedia Laboratories were maintained in appropriate media (MRS broth for bacteria and potato dextrose agar for fungus and yeast) for five days at 35-37°C in a shaking incubator. The spores were harvested in 0.1% Tween 80 and were approximately adjusted to 10^7 spores ml^{-1}, whereas the bacterial colony was diluted to 10^6-10^7 ml^{-1}. The plant protein sources were sterilised with moisture of 60-65% and were subsequently inoculated with respective microbial suspension (5%) and the flasks were incubated at 35-37°C in a shaking incubator for three days. The fungal and yeast fermentation was carried out in 500 ml Erlenmeyer flask covered with cotton plugs to facilitate air transfer whereas the bacterial fermentation was carried out in anaerobic condition by vacuum sealing. Simultaneously, another set of ingredients were allowed to ferment naturally without any inoculum at an ambient temperature. All the treatments were carried out in six replications and the final fermented products were collected after oven drying (40-50°C).

Laboratory analysis

The amino acids were analysed using pre-column HPLC gradient system (Shimadzu Corp, LC-30AD) after hydrolysing the samples with 6N hydrochloric acid in a sealed tube for 22 h at 110°C in a vacuum oven (Finlayson, 1964). The acid was drained using vacuum rotary evaporator (IKA, Model No: RE 10 C S84) and the residue was brought into diluent (0.1N hydrochloric acid) and then filtered using 0.2 µm membrane syringe filter. YMC-Triart C18, RRH (1.8 µm, 2.1x100 mm dimension) column was used to separate the amino acids after derivatisation with mercaptopropionic acid, O-pthaladehyde and fluorenylmethoxycarbonyl chloride under gradient elution using phosphate buffer (20 mm; buffer A) and combination of acetonitrile:methanol: water (45:40:15; buffer B) at a flow rate of 0.3 ml min^{-1}. The gradient was changed by increasing buffer B concentration at the rate of 11-13% at 3 min, 31% at 5 min, 37% at 15 min, 70% at 20 min, 100% at 25-28 min and finally, 11% at 30 min. Amino acids were identified and quantified by fluorescent detector (RF-20AXS) using amino acid mixer as an external standard (Sigma Aldrich, Cat. No: AAS18-5ml). Tryptophan being labile to acid hydrolysis, it was measured after alkali hydrolysis by spectrophotometric method (Sastry and Tammuru, 1985) at 500 nm. The essential amino acid index (EAAI) was calculated based on the amino acid requirements of *P. vannamei* (Akiyama *et al.,* 1991).

Fiber fractions namely neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose and lignin content of selected plant protein sources were estimated as per the method of Van Soest (1963). Anti-nutritional factors such as trypsin inhibitor (Kakade *et al*., 1974), saponin (AOAC, 1997), phytic acid (Davis and Reid, 1979), tannin (Vannilin HCl), glucosinolate (McGhee *et al*., 1965) and guar gum (Das *et al*., 1977) were analysed by standard methods.

Statistical analysis

The data was statistically evaluated by one way ANOVA using SPSS version 17.0. The post-hoc analysis was done using least significance difference. Comparison of means was carried out at 5% significance level ($p<0.05$).

Results and discussion

The nutritional quality of agricultural residues was improved by solid state fermentation through the reduction of undesired substances effectively compared to other detoxification process (Shi *et al.,* 2015). The effect of different inoculums on essential amino acids and microbial degradation of anti-nutrients by natural, bacterial, fungal and yeast fermentation of various plant protein sources are discussed here.

Effect of SSF on essential amino acids (EAA) and essential amino acid index (EAAI)

Generally, shrimp feeds are formulated in terms of crude protein (CP) and the quality of protein sources is expressed as the amount of essential amino acids in the CP. This information is important to formulate balanced cost effective feeds. Changes in the essential amino acid (EAA) contents induced by solid state fermentation are given in Table 1. The total EAA content of SBM, GNC, RSM, SFC and GRM was significantly increased ($p<0.05$) to a range of 20.19-24.89, 16.62-19.56, 17.33-21.40, 15.98-16.92 and 22.10-24.18% after fermentation compared to the respective control (19.44, 15.48, 16.90, 14.32 and 21.29%).

Table 1. Effect of solid state fermentation on essential amino acid contents (% dry matter basis) of plant protein sources (n=6; mean±SD)

Treatments	Essential amino acids (EAA)									
	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	TRP	VAL
SBM	3.00^{e}±0.22	1.75^{c}±0.17	2.73^{e}±0.06	3.93^{c}±0.08	1.25^{d}±0.16	0.74^{c}±0.11	2.02^{d}±0.10	1.71^{c}±0.12	0.67^{b}±0.05	1.63^{c}±0.07
NSBM	3.67^{b}±0.25	2.06^{a}±0.14	3.16^{a}±0.13	4.32^{a}±0.03	1.72^{b}±0.19	0.88^{b}±0.08	2.30^{b}±0.03	2.01^{a}±0.10	0.77^{a}±0.03	2.00^{a}±0.10
BSBM	2.87^{c}±0.46	1.90^{b}±0.06	2.72^{c}±0.12	4.01^{b}±0.08	1.33^{d}±0.09	0.80^{bc}±0.05	2.20^{c}±0.05	1.81^{bc}±0.10	0.71^{b}±0.02	1.84^{b}±0.07
FSBM	4.07^{a}±0.30	1.95^{ab}±0.10	2.90^{b}±0.05	4.01^{b}±0.06	4.01^{a}±0.22	0.99^{a}±0.07	2.52^{a}±0.11	1.91^{ab}±0.05	0.77^{a}±0.04	1.76^{b}±0.11
YSBM	3.85^{ab}±0.15	1.98^{ab}±0.13	2.95^{b}±0.13	3.04^{d}±0.14	1.47^{b}±0.02	0.84^{b}±0.03	2.47^{a}±0.15	1.93^{a}±0.05	0.79^{a}±0.05	1.82^{b}±0.05
GNC	3.04^{d}±0.21	0.91^{b}±0.10	1.36^{e}±0.06	1.09^{e}±0.02	1.42^{d}±0.15	0.56^{d}±0.16	3.00^{c}±0.07	0.95^{d}±0.07	0.43^{d}±0.02	2.71^{c}±0.05
NGNC	3.75^{a}±0.09	1.12^{a}±0.08	1.51^{bc}±0.23	1.27^{ab}±0.09	1.73^{bc}±0.17	0.71^{bc}±0.13	3.53^{a}±0.19	1.07^{c}±0.10	0.50^{bc}±0.03	2.71^{c}±0.01
BGNC	3.21^{c}±0.02	1.00^{b}±0.02	1.39^{bc}±0.02	1.22^{b}±0.03	1.54^{cd}±0.04	0.61^{cd}±0.00	3.16^{b}±0.02	1.07^{c}±0.04	0.49^{a}±0.03	2.95^{a}±0.03
FGNC	3.58^{b}±0.18	1.09^{a}±0.09	1.52^{b}±0.07	1.11^{c}±0.04	3.51^{a}±0.30	1.03^{a}±0.13	3.10^{bc}±0.05	1.31^{a}±0.09	0.52^{ab}±0.03	2.79^{b}±0.04
YGNC	3.77^{a}±0.13	1.15^{a}±0.06	1.67^{a}±0.03	1.33^{a}±0.02	1.77^{b}±0.10	0.76^{b}±0.10	3.64^{a}±0.04	1.18^{b}±0.04	0.53^{a}±0.01	2.73^{c}±0.01
RSM	3.62^{c}±0.14	1.73^{c}±0.15	1.56^{d}±0.04	2.04^{d}±0.11	1.10^{d}±0.15	0.88^{d}±0.11	0.99^{c}±0.15	2.15^{c}±0.06	0.47^{c}±0.02	2.36^{c}±0.11
NRSM	3.93^{a}±0.25	1.97^{a}±0.09	1.74^{a}±0.02	2.34^{b}±0.06	1.31^{c}±0.08	1.04^{c}±0.06	1.23^{b}±0.11	2.42^{a}±0.03	0.53^{a}±0.01	2.68^{b}±0.06
BRSM	3.65^{c}±0.04	1.82^{b}±0.03	1.54^{d}±0.03	2.21^{c}±0.03	1.11^{d}±0.03	0.92^{d}±0.02	0.99^{c}±0.02	2.25^{b}±0.01	0.52^{b}±0.01	2.32^{c}±0.06
FRSM	3.82^{ab}±0.15	1.78^{bc}±0.18	1.66^{b}±0.05	2.56^{a}±0.09	3.01^{a}±0.16	1.41^{a}±0.10	1.63^{a}±0.18	2.28^{b}±0.05	0.53^{a}±0.02	2.72^{b}±0.09
YRSM	3.76^{bc}±0.05	1.75^{bc}±0.06	1.60^{c}±0.04	2.35^{b}±0.04	2.06^{b}±0.05	1.17^{b}±0.07	1.24^{b}±0.04	2.15^{c}±0.08	0.53^{a}±0.01	2.86^{a}±0.05
SFC	1.62^{b}±0.12	0.47^{c}±0.06	3.37^{b}±0.06	1.46^{c}±0.08	1.18^{c}±0.19	1.70^{b}±0.16	1.60^{b}±0.09	1.02^{c}±0.11	0.42^{c}±0.04	1.48^{b}±0.10
NSFC	1.97^{a}±0.16	0.66^{a}±0.05	3.70^{a}±0.03	1.69^{b}±0.04	1.45^{c}±0.07	1.97^{a}±0.11	2.02^{a}±0.19	1.23^{b}±0.05	0.46^{ab}±0.01	1.72^{a}±0.04
BSFC	1.68^{b}±0.03	0.49^{c}±0.01	3.48^{b}±0.05	1.51^{c}±0.02	2.14^{a}±0.13	1.79^{b}±0.04	1.69^{b}±0.01	1.18^{b}±0.02	0.48^{a}±0.01	1.56^{b}±0.06
FSFC	1.88^{a}±0.10	0.56^{b}±0.06	3.37^{b}±0.06	1.87^{a}±0.11	2.31^{a}±0.28	1.77^{b}±0.09	1.72^{b}±0.05	1.50^{a}±0.13	0.44^{bc}±0.03	1.49^{b}±0.10
YSFC	1.75^{b}±0.04	0.52^{bc}±0.02	3.11^{c}±0.29	1.65^{b}±0.13	1.74^{b}±0.45	1.70^{b}±0.05	1.64^{b}±0.06	1.27^{b}±0.07	0.46^{ab}±0.02	1.58^{b}±0.12
GRM	6.19^{b}±0.19	1.30^{d}±0.10	2.15^{b}±0.10	1.05^{d}±0.13	2.06^{c}±0.16	0.65^{b}±0.15	2.20^{c}±0.06	2.47^{b}±0.09	0.79^{d}±0.04	2.43^{c}±0.06
NGRM	6.24^{b}±0.02	1.42^{bc}±0.06	2.37^{a}±0.07	1.19^{bc}±0.09	2.09^{c}±0.03	0.70^{b}±0.02	2.57^{a}±0.10	2.48^{b}±0.03	0.81^{cd}±0.01	2.61^{ab}±0.05
BGRM	6.27^{b}±0.04	1.38^{cd}±0.06	2.20^{b}±0.05	1.10^{cd}±0.05	2.12^{bc}±0.04	0.73^{b}±0.06	2.26^{c}±0.03	2.63^{a}±0.12	0.87^{b}±0.06	2.54^{bc}±0.07
FGRM	6.70^{a}±0.18	1.52^{ab}±0.13	2.18^{b}±0.09	1.27^{ab}±0.06	3.33^{a}±0.17	1.08^{a}±0.10	2.27^{c}±0.09	2.49^{b}±0.08	0.86^{bc}±0.04	2.48^{c}±0.12
YGRM	6.70^{a}±0.07	1.54^{a}±0.06	2.25^{b}±0.09	1.32^{a}±0.07	2.23^{b}±0.06	1.18^{a}±0.03	2.44^{b}±0.07	2.62^{a}±0.04	0.94^{a}±0.05	2.65^{a}±0.09

N, B, F and Y indicate natural, bacterial, fungal and yeast fermented samples of respective ingredients

Means bearing same superscript in a column between raw and respective fermented samples do not differ significantly ($p>0.05$)

Natural fermentation showed significant ($p<0.05$) increase in all the EAA compared to the respective control. The increment was more in ARG and VAL in SBM, ARG and LYS in GNC, PHE in RSM, ARG, HIS, LYS, PHE and THR in SFC. The increment of EAA in naturally fermented palm kernel meal and copra meal was also previously reported by Dairo and Fasuyi (2007), but in contrast, Osman (2011) reported a reduced level of certain EAA in natural fermented pearl millet which might be due to the short fermentation period (12 h). The fungus, *A. niger* markedly increased the LYS (2.31-4.01%) irrespective of the ingredients tested. The higher increase of LYS might be due to the higher content of this amino acid in *A. niger* (6.5-7.8%) compared to other microbial species (bacterial species contain about 4.5-5.8%; Ravindra, 2000). The improvement was the highest for ARG in YSBM, LYS in YGNC and YGRM, LEU in YRSM and YSFC than other amino acids in yeast fermentation. Increased EAA contents were also documented in SBM with *Bacillus subtilis* (Imelda *et al.,* 2008) and *S. cerevisiae* (Sharawy *et al.,* 2016), RSM with *A. niger* (Shi *et al.,* 2015). However, Hong *et al.* (2004) reported that the fermentation of SBM by *A. oryzae* had no effect on the EAA profiles.

The variations in the increase of essential amino acids during fermentation might be due to the inoculum itself (Shankman, 1943; Christias *et al.*, 1975; Watson, 1976). The increased microbial growth during fermentation resulted in the increase of own amino acid content, which might have reflected in the fermented samples. Microorganism utilised carbohydrates as a source of energy and the bio-conversion of such carbohydrates into microbial protein by intermediary metabolism might also be responsible for the increase observed in the amino acid profiles (Imelda *et al.,* 2008). The increase of amino acids in fermentation might be due to the nitrogen fixing ability, as earlier reported by Lipman (1911) that various strains of fungus, *A. niger* and yeast, *S. cervesiae* assimilated 0.63-2.23 and 0.76-1.74 mg 100 cc^{-1} nitrogen respectively. Later Schober (1930) also confirmed the above result for *A. niger* who reported that the fixation was much higher (4 mg 100 cc^{-1}) than the previous reports. But the above results failed to corroborate the results of Allison (1934) who reported negative results for the same species. Recently, Sharma and Kumawat (2011) further confirmed the possible role of *A. niger* and *B. japonicum* isolates on plant growth in terms of nitrogen fixation, which clearly indicated the role of *A. niger* in nitrogen

fixation. It would seem that the nitrogen fixation was probably limited and the quantity fixed may be less, but it is of considerable importance for the microbial growth as well as the production of metabolites like amino acids. But the phenomena need to be ascertained by further investigations.

Furthermore, the changes in other nutrients and also dry matter loss could also be a possible reason for the increase of amino acids observed in the present study. About 5-14% dry matter loss was seen in the present work, which was comparatively less than certain physical and chemical methods (Shi *et al.,* 2015). Almost a similar tendency was noticed for nonessential amino acids (NAA) and the reduction of certain NAA (Table 2) might be attributed to the utilisation of particular amino acids for growth and production of enzymes and other organic compounds by the microorganisms (Imelda *et al.,* 2008).

The quality of protein can be assayed based on the amino acid composition but its suitability to the candidate species depends on its amino acid requirements. Earlier, chemical score was calculated by taking the most limiting amino acid into consideration. Later, EAAI was calculated based on the overall essential amino acid requirements (Akiyama *et al*., 1991). The quality of fermented ingredients with different inoculums was assessed by calculating EAAI for *P. vannamei* for optimising the fermentation method. The fungal fermentation resulted in the best improvement of most limiting indispensable amino acids like LYS, MET and TRP which in turn resulted in the improvement of EAAI for *P. vannamei*. This higher improvement of EAA might be due to its higher contents in *A. niger* compared to other inoculums used in the present study. The same was confirmed by Christias *et al.* (1975) who reported that the total EAA content was 102.24 µM 100 mg dry weight^{-1} in *A. niger,* whereas it was 8.81 µM 100 mg dry weight^{-1} in *S. cerevisea* (Watson, 1976) and 0.10 µM 100 mg dry weight^{-1} in *Lactobacillus* sp. (Shankman, 1943). The reflection of higher content of EAA of fungus in the respective treated samples might be responsible for such improvement in EAAI than other treatments. Among the ingredients tested, the lowest EAAI was observed in FGRM followed by FSFC and FGNC (Fig. 1). The best improvement of EAAI was observed with fungal fermentation in FRSM (0.824±0.015 to 0.916±0.003). The results clearly indicate

Table 2. Effect of solid state fermentation on essential amino acid contents (% dry matter basis) of plant protein sources (n=6; mean±SD)

Treatments	Non-essential amino acids (NAA)							
	ALA	ASP	CYT	GLU	GLY	PRO	SER	TYR
SBM	3.32^{b}±0.11	5.03^{c}±0.07	0.80^{c}±0.06	7.95^{b}±0.10	1.07^{bc}±0.09	2.74^{bc}±0.10	2.51^{d}±0.19	2.34^{c}±0.10
NSBM	3.74^{a}±0.08	5.58^{a}±0.09	0.98^{b}±0.02	8.17^{a}±0.05	1.17^{a}±0.11	2.88^{a}±0.11	3.28^{a}±0.09	2.47^{ab}±0.07
BSBM	2.94^{c}±0.15	5.22^{b}±0.09	0.85^{c}±0.03	8.20^{a}±0.07	1.03^{c}±0.04	2.79^{ab}±0.04	2.62^{d}±0.12	2.54^{a}±0.09
FSBM	3.42^{b}±0.10	5.25^{b}±0.08	1.07^{a}±0.12	8.02^{b}±0.11	1.19^{a}±0.12	2.67^{c}±0.12	3.13^{b}±0.15	2.43^{abc}±0.08
YSBM	3.65^{a}±0.10	5.54^{a}±0.11	1.00^{ab}±0.05	8.26^{a}±0.13	1.14^{ab}±0.02	2.82^{ab}±0.04	2.74^{c}±0.09	2.37^{bc}±0.20
GNC	2.02^{c}±0.05	3.24^{e}±0.05	0.61^{d}±0.13	6.27^{c}±0.19	2.03^{b}±0.16	1.68^{c}±0.11	2.13^{c}±0.18	3.54^{b}±0.06
NGNC	2.16^{b}±0.01	3.92^{b}±0.06	1.01^{b}±0.09	7.36^{a}±0.10	2.36^{a}±0.05	1.64^{c}±0.02	2.65^{a}±0.05	3.59^{b}±0.05
BGNC	2.05^{c}±0.01	3.42^{d}±0.06	0.77^{c}±0.04	6.40^{d}±0.06	2.36^{a}±0.21	1.59^{c}±0.06	2.53^{ab}±0.09	3.56^{b}±0.08
FGNC	2.14^{b}±0.09	4.19^{a}±0.03	1.11^{a}±0.09	7.09^{b}±0.15	2.44^{a}±0.12	2.20^{b}±0.13	2.48^{b}±0.15	3.56^{b}±0.07
YGNC	2.29^{a}±0.04	3.72^{c}±0.07	0.93^{b}±0.07	6.57^{c}±0.12	2.32^{a}±0.23	2.43^{a}±0.03	1.82^{d}±0.16	3.81^{a}±0.06
RSM	2.69^{c}±0.09	2.24^{d}±0.05	1.12^{d}±0.11	5.10^{b}±0.07	3.21^{b}±0.05	2.32z^{c}±0.09	2.49^{b}±0.09	1.72^{a}±0.06
NRSM	2.83^{ab}±0.10	2.65^{a}±0.05	1.59^{c}±0.03	5.30^{a}±0.09	3.35^{a}±0.09	2.52^{a}±0.08	2.21^{c}±0.06	1.72^{a}±0.09
BRSM	2.61^{d}±0.08	2.32^{c}±0.01	1.04^{c}±0.02	5.10^{b}±0.11	3.22^{b}±0.15	2.39^{bc}±0.12	2.44^{b}±0.04	1.69^{a}±0.10
FRSM	2.84^{a}±0.08	2.54^{b}±0.03	1.79^{b}±0.09	5.11^{b}±0.05	3.31^{ab}±0.15	2.54^{a}±0.05	2.67^{a}±0.09	1.82^{a}±0.07
YRSM	2.76^{bc}±0.06	2.57^{b}±0.08	2.02^{a}±0.04	5.25^{a}±0.11	3.00^{c}±0.05	2.47^{ab}±0.07	2.46^{b}±0.10	1.74^{a}±0.02
SFC	1.09^{c}±0.07	1.63^{c}±0.08	1.23^{ab}±0.08	6.95^{a}±0.09	1.09^{d}±0.09	1.33^{b}±0.10	1.40^{b}±0.06	1.64^{bc}±0.05
NSFC	1.18^{bc}±0.18	1.93^{a}±0.01	1.09^{d}±0.06	4.89^{a}±3.24	1.23^{bc}±0.10	1.24^{c}±0.02	1.70^{a}±0.06	1.58^{c}±0.07
BSFC	1.06^{c}±0.03	1.71^{b}±0.06	1.16^{c}±0.02	6.94^{a}±0.07	1.55^{a}±0.15	1.16^{d}±0.04	1.11^{c}±0.05	1.64^{bc}±0.02
FSFC	1.24^{bc}±0.08	1.89^{a}±0.07	1.29^{a}±0.07	6.98^{a}±0.09	1.32^{b}±0.10	1.57^{a}±0.12	1.51^{b}±0.12	1.71^{b}±0.09
YSFC	1.52^{a}±0.07	1.70^{b}±0.09	1.18^{bc}±0.03	7.18^{a}±0.12	1.16^{cd}±0.01	1.40^{b}±0.08	1.17^{c}±0.14	1.80^{a}±0.13
GRM	1.61^{d}±0.07	4.31^{c}±0.05	0.71^{c}±0.15	6.54^{b}±0.07	2.77^{c}±0.12	2.60^{bc}±0.07	3.59^{c}±0.21	1.91^{c}±0.09
NGRM	1.92^{b}±0.07	4.63^{a}±0.03	0.90^{b}±0.01	6.61^{b}±0.02	3.01^{b}±0.05	2.79^{a}±0.03	4.14^{c}±0.10	2.25^{a}±0.16
BGRM	1.77^{c}±0.06	4.07^{d}±0.04	0.85^{b}±0.03	6.58^{b}±0.12	2.46^{d}±0.08	2.84^{a}±0.05	3.81^{d}±0.08	2.05^{bc}±0.11
FGRM	1.93^{b}±0.10	4.38^{c}±0.05	1.18^{a}±0.15	6.81^{a}±0.05	3.22^{a}±0.15	2.65^{b}±0.07	4.78^{a}±0.18	2.18^{ab}±0.07
YGRM	2.29^{a}±0.14	4.55^{b}±0.10	0.93^{b}±0.04	6.77^{a}±0.05	3.10^{b}±0.03	2.54^{c}±0.08	4.44^{b}±0.12	2.18^{ab}±0.16

N, B, F and Y indicate natural, bacterial, fungal and yeast fermented samples of respective ingredients

Means bearing same superscript in a column between raw and respective fermented samples do not differ significantly ($p>0.05$)

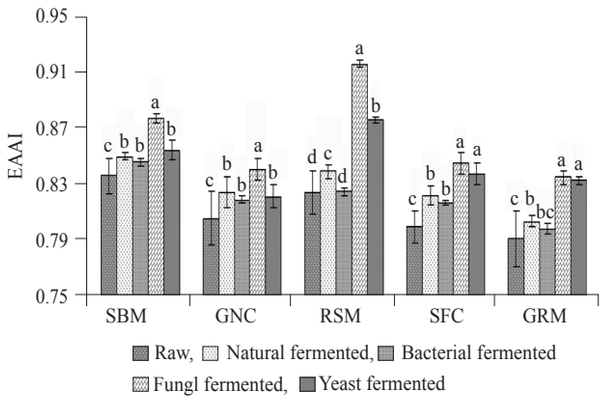

Fig. 1. Effect of solid state fermentation on essential amino acid index (EAAI) in plant protein sources

that the amino acid composition varies not only with the inoculums used for fermentation but also based on the substrate.

Effect of SSF on fiber fractions

The effect of different methods of SSF on fiber fractions of plant protein sources are shown in Table 3. The level of NDF was 11.98, 21.54, 26.58, 43.86 and 19.71% in SBM, GNC, RSM, SFC and GRM and was reduced to a range of 11.47-11.85, 20.95-21.29, 25.30-25.33, 40.36-41.09 and 11.59-15.34% by fungal and yeast fermentation, respectively. The percentage of reduction was found to be highest in GRM (22-41%) compared to other ingredients (1-8%). Similarly, fungal and yeast fermented GRM showed the highest ADF reduction (around 15%), whereas it was between 0.2 and 5.4% for other ingredients. This might be due to the differences in the production of fibrolytic enzymes according to the substrate and microorganism during fermentation (Shi *et al*., 2015).

Fungal fermentation showed almost similar reduction of cellulose (19.80-26.88%) among all the ingredients tested but the reduction ($p<0.05$) varied between ingredients in yeast fermentation (5.25-17.02%). It clearly indicates that the production of individual fibrolytic enzymes had differed according to the strain and substrate used for fermentation. Hemicellulose was reduced by 2.5-2.7% in SBM, 2.0-6.3% in GNC, 6.4-11.0% in RSM, 10.1-21.8% in SFC and 25.7-62.0% in GRM respectively.

Table 3. Effect of solid state fermentation on fiber fractions (% dry matter basis) of plant protein sources (n=6; mean ± SD)

Treatments	Fiber fractions				
	NDF	ADF	Cellulose	Hemicellulose	Lignin
SBM	11.98^{c}±0.16	7.82^{b}±0.25	6.96^{a}±0.21	4.15^{c}±0.30	0.86^{a}±0.05
NSBM	13.25^{b}±0.17	8.09^{a}±0.04	6.99^{a}±0.07	5.16^{b}±0.21	0.92^{a}±0.04
BSBM	15.85^{a}±0.06	8.31^{a}±0.06	7.01^{a}±0.17	7.54^{a}±0.00	0.90^{a}±0.07
FSBM	11.47^{d}±0.08	7.39^{c}±0.17	5.58^{c}±0.24	4.01^{c}±0.01	0.73^{b}±0.04
YSBM	11.85^{c}±0.23	7.81^{b}±0.41	6.21^{b}±0.55	4.05^{c}±0.42	0.85^{a}±0.12
GNC	21.54^{b}±0.23	13.90^{b}±0.46	8.75^{b}±0.48	7.64^{b}±0.79	5.15^{a}±0.01
NGNC	24.69^{a}±0.06	16.19^{a}±0.08	10.51^{a}±0.01	8.50^{a}±0.14	5.28^{a}±0.12
BGNC	24.96^{a}±0.35	16.43^{a}±0.43	10.79^{a}±0.40	8.54^{a}±0.78	5.22^{a}±0.03
FGNC	20.95^{c}±0.33	13.80^{b}±0.37	6.85^{d}±0.33	7.15^{b}±0.67	5.10^{a}±0.09
YGNC	21.29^{bc}±0.58	13.87^{b}±0.61	7.79^{c}±0.49	7.42^{b}±0.66	5.12^{a}±0.27
RSM	26.58^{c}±0.11	19.34^{b}±0.33	8.62^{b}±0.54	7.25^{bc}±0.36	10.72^{a}±0.21
NRSM	29.00^{a}±0.33	20.78^{a}±0.09	9.48^{a}±0.42	8.22^{a}±0.29	10.95^{a}±0.49
BRSM	28.14^{b}±0.42	20.74^{a}±0.42	9.49^{a}±0.20	7.40^{b}±0.60	10.90^{a}±0.29
FRSM	25.30^{d}±0.70	18.51^{d}±0.02	7.63^{c}±0.06	6.80^{cd}±0.68	8.48^{c}±0.01
YRSM	25.33^{d}±0.03	18.90^{c}±0.24	8.62^{b}±0.40	6.43^{d}±0.21	9.58^{b}±0.10
SFC	43.86^{c}±0.52	27.59^{c}±0.20	19.77^{b}±0.16	16.27^{b}±0.44	7.82^{c}±0.05
NSFC	46.17^{a}±0.15	29.28^{a}±0.17	20.11^{a}±0.27	16.89^{a}±0.32	8.95^{a}±0.09
BSFC	45.65^{b}±0.48	29.19^{a}±0.13	20.06^{a}±0.13	16.46^{ab}±0.47	8.64^{b}±0.11
FSFC	40.36^{e}±0.37	25.72^{d}±0.29	15.60^{d}±0.27	14.64^{c}±0.56	7.69^{c}±0.09
YSFC	41.09^{d}±0.22	28.40^{b}±0.37	18.72^{c}±0.17	12.69^{d}±0.59	8.74^{b}±0.23
GRM	19.71^{b}±0.30	9.01^{b}±0.17	7.90^{b}±0.25	10.70^{b}±0.53	1.11^{b}±0.09
NGRM	20.85^{a}±0.22	9.81^{a}±0.17	8.08^{ab}±0.17	11.53^{a}±0.06	1.38^{a}±0.15
BGRM	20.86^{a}±0.65	10.08^{a}±0.92	8.44 ^{a}±0.90	11.25^{a}±0.31	1.28^{ab}±0.33
FGRM	11.59^{d}±0.09	7.66^{c}±0.11	5.72^{d}±0.23	3.93^{d}±0.05	0.44^{c}±0.04
YGRM	15.34^{c}±0.40	7.70^{c}±0.04	6.53^{c}±0.05	7.96^{c}±0.43	0.47^{c}±0.04

N, B, F and Y indicate natural, bacterial, fungal and yeast fermented samples of respective ingredients
Means bearing same superscript in a column between raw and respective fermented samples do not differ significantly ($p>0.05$)
NDF - Neutral detergent fiber, ADF - Acid detergent fiber

Fungal fermented RSM showed significant reduction ($p<0.05$) of lignin (10.72 to 8.48%) whereas it was not much affected in other ingredients tested. The reduction in fiber fractions after fermentation was associated with the production of fibrolytic enzymes by the inherent microorganisms (Shi *et al.,* 2015). Whereas in bacterial and natural fermentation, fiber fractions were not reduced, which might be due to the lack of production of respective enzymes by the microorganisms or unsuitable selection of microorganisms responsible for fiber degradation based on substrate or the utilisation of easily digestible soluble carbohydrates by the growing microorganism and leaving the indigestible fiber content (Amanullah *et al*., 2014).

Effect of SSF on anti-nutrients

The level of anti-nutrients of both raw and fermented plant protein sources are presented in Table 4. Trypsin inhibitor was identified only in SBM and GRM and was reduced in all the fermentation methods. The reduction of trypsin inhibitor was mainly attributed to the application of heat during autoclaving (Hong *et al*., 2004), but its reduction in natural fermented samples indicated the role of microorganisms in degrading trypsin inhibitor. Roychaudhuri *et al*. (2004) inferred that the trypsin inhibitor in SBM belonging to the family of anti-parallel β-sheet proteins renature and thus cannot be completely removed by processing methods. Though, trypsin inhibitor in GRM also belongs to the same family, its level was reduced to below detectable range after treatment and the same was supported by Mubarak (2005) in mung bean.

Saponin was significantly ($p<0.05$) reduced by 65.8-79.1% in SBM, 38.6-49.0% in GNC, 24.8-67.7% in SFC and 56.7-62.2% in GRM due to solid state fermentation. The reduction of saponin content might be due to the degradation of saponin into sapogenin and sugar moieties by the microbial enzyme glycosidase (Makkar and Becker, 1997). The phytic acid reduction was better with bacterial fermentation (40.5-67.6%) compared to other treatments (17.9-53.8%). The reduction of phytic acid was due to the activity of the endogenous phytase enzyme during fermentation, which hydrolyse the phytic acid into inositol and orthophosphate (Reddy and Peirson, 1994). The reduction of tannin content was found to be high in GNC (74.9-83.5%), whereas it was <42.7% for other ingredients. Production of enzyme tannase or

Table 4. Effect of solid state fermentation on the level of anti-nutrients (mg 100 g dry matter basis^{-1}) of plant protein sources (n=6; mean ± SD)

Treatments	Anti-nutrients					
	Trypsin inhibitor	Saponin	Phytic acid	Tannin	Glucosinolates	Guar gum (%)
SBM	241.05^{a}±2.99	1003.16^{a}±0.50	1335.67^{a}±23.23	-	-	-
NSBM	87.15^{b}±98.77	343.14^{b}±14.61	1029.41^{b}±44.82	-	-	-
BSBM	nd	259.43^{c}±38.67	611.47^{e}±63.23	-	-	-
FSBM	13.97^{c}±1.95	209.52^{d}±4.81	653.27^{d}±17.30	-	-	-
YSBM	18.15^{c}±3.82	229.58^{d}±4.78	792.31^{c}±37.25	-	-	-
GNC	-	736.17^{a}±58.10	1033.16^{a}±25.70	1756.02^{a}±85.24	-	-
NGNC	-	451.99^{b}±69.02	847.76^{b}±64.68	417.54^{b}±33.84	-	-
BGNC	-	381.15^{c}±33.84	614.49^{d}±38.49	382.95^{b}±10.29	-	-
FGNC	-	375.66^{c}±38.40	646.49^{d}±29.75	288.95^{c}±7.58	-	-
YGNC	-	429.48^{bc}±21.48	707.93^{c}±59.08	440.96^{b}±56.60	-	-
RSM	-	-	2745.44^{a}±134.03	889.43^{a}±29.88	313.16^{a}±11.77	-
NRSM	-	-	1850.99^{b}±110.92	791.07^{b}±30.52	273.95^{b}±15.31	-
BRSM	-	-	890.76^{d}±22.93	667.54^{c}±2.87	238.94^{c}±7.78	-
FRSM	-	-	897.28^{d}±22.93	509.89^{d}±26.24	182.48^{e}±7.77	-
YRSM	-	-	1216.72^{c}±98.34	510.19^{d}±15.20	210.70^{d}±13.92	-
SFC	-	641.52^{a}±39.57	-	878.55^{a}±17.70	-	-
NSFC	-	482.25^{b}±2.3.70	-	581.74^{c}±34.40	-	-
BSFC	-	254.16^{c}±37.89	-	694.54^{b}±49.04	-	-
FSFC	-	217.07^{d}±23.68	-	610.36^{c}±9.23	-	-
YSFC	-	207.01^{d}±18.99	-	610.86^{c}±23.92	-	-
GRM	81.06^{a}±8.37	2552.82^{a}±49.21	2567.49^{a}±156.44	392.33^{a}±7.96	-	10.99^{a}±0.53
NGRM	38.90^{b}±6.76	1016.97^{c}±70.69	1966.84^{b}±16.98	369.64^{b}±14.77	-	10.16^{a}±1.25
BGRM	nd	1001.90^{c}±66.03	1113.69^{d}±15.76	356.22^{c}±6.25	-	10.22^{a}±0.58
FGRM	nd	964.72^{c}±33.37	1187.15^{d}±27.57	333.53^{d}±12.06	-	10.17^{a}±0.58
YGRM	nd	1105.00^{b}±52.02	1632.30^{c}±27.19	320.70^{d}±0.96	-	10.27^{a}±0.87

N, B, F and Y indicate natural, bacterial, fungal and yeast fermented samples of respective ingredients
Means bearing same superscript in a column between row and respective fermented samples do not differ significantly ($p>0.05$)
nd: Not detected

microbial phenyl oxidase action might be responsible for the reduction of tannin content in the treated samples (Emambux and Taylor, 2003).

Glucosinolates were reduced by 41.7% by *A. niger* and the reduction was 12.5-32.7% with other treatments. Reduced glucosinolates mainly attributed to the utilisation of glucose and sulphur moieties during fermentation (Shi *et al*., 2015). The reduction of gum content in guar meal due to fermentation was comparatively lower than other anti-nutrients.

All the four fermentation methodologies showed a positive effect on EAA and better EAAI was observed with fungal fermentation. Anti-nutrients were also effectively reduced by the specific inoculums rather than the natural fermentation. The plant ingredients inoculated with fungal and yeast showed reduction of fiber fractions. From the present investigation, it was concluded that specific inoculum fermentation especially fungal and yeast methods can be considered as a potential processing technique to produce nutrient enriched products which are of industrial importance. This data will pave the way for higher fishmeal replacement using fermented ingredients in shrimp feed formulations.

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