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Bio-processing of low-cost sesame oil cake for nutritional enhancement of carp diet

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ABSTRACT

Utilization of inexpensive oil cakes in fish diet formulations is essential to counteract problems related to the shortage of quality fish meal and the increase in prices of conventional fish feed ingredients like soybean meal, groundnut oil cake and mustard oil cake. This study determined the extent to which the low-cost sesame oil cake (SOC) could be fermented through the solid-state fermentation (SSF) process and used in the formulation of diets for carp. Autoclaved dough made from SOC powder with 60% moisture content was fermented separately with three probiotics viz. aqualact, rhodomax and uni-nutrich plus by using plastic trash cans as fermentation chamber and by using inoculum of probiotics powder, jaggery and water in the proportion of 5 g : 25 g :160 ml per kg of oil cake substrate for seven days. Fermented feed samples were collected in each 24 h interval, oven dried at 60±5°C up to constant moisture level; analyzed for their nutrients (crude protein, crude fiber) and anti-nutritional factors (ANFs) composition. The best among the fermented products at a particular fermentation duration of a particular oil cake was ascertained by taking their proximate composition into consideration, i.e., the maximum crude protein content, the minimum crude fiber content, and complete elimination of ANFs (tannin and phytic acid). Fermented sesame oil cake was found to be a viable alternative to costlier fish meal, soybean meal, groundnut oil cake, and mustard oil cake in the formulation of carp diets. Further studies are required to carry out field trials and SSF of other low-cost non-conventional feedstuffs with various other commercial feed probiotics available in the market.

Key words: Carp, diet, probiotics, sesame oil cake, solid state fermentation

INTRODUCTION

The scope for utilizing low-cost plant-derived protein sources and energy feedstuffs in fish feed formulation has been expanded in India because of the availability of large quantities of agro-based materials that are unusable to human beings. Moreover, aquaculture in India largely depends on the culture of herbivores or omnivore carp species. Therefore, the use of plant-based fish feed could be justified in developing countries like India. Wide use and acceptability of plant-derived protein sources as alternatives to a fish meal have been constrained due to their high fiber content, imbalanced amino acid profiles, and presence of some anti-nutritional factors (ANFs), such as tannin, phytic acid, trypsin inhibitor, lectins, saponins and many more (Francis et al., 2001).

It has been postulated that the ANFs can seriously reduce the feed nutritional value of different plant-derived materials unless destroyed or deactivated (Liener, 1975; Hossain and Janucey, 1989). The common detoxification methods to overcome most of the anti-nutritional factors are inactivation by heat and/or soaking in water

(Mukhopadhyay and Ray, 1996, 1999a,b,c). However, these physical processes are not conclusive in removing the deleterious effects of the wide variety of ANFs. Heat damage affects the usefulness of plant ingredients when fed to fish (Glencross et al., 2004). Nutritional loss of some amino acids through heat treatment and the influence of protein, carbohydrate, and moisture on Malliard reactions is well known (Anderson et al., 1993; Oste and Sjodin, 1984). Soaking in water may be effective in removing many ANFs, however, water-soluble nutrients may leach out by this process. Apart from the traditional physical processes, microbial deactivation (by solid-state fermentation, SSF) has been evaluated in the present study for the removal of ANFs in plant feedstuffs. SSF is a bioprocess in which microorganisms are grown on solid substances with low or minimum water levels (Cannel and Moo-Young, 1980; Pandey, 1992, 1994; Van de Lagemaat and Pyle, 2001). It provides conditions under which microorganisms grow in the wild, so it may be considered more natural than other techniques (Hesseltine, 1987). The major advantages of utilization of low-cost plant ingredients as substrates in SSF might be its great economic feasibility and as a way of nutrient recycling. The sustainability of the aquaculture industry depends on the availability of low-cost and high-quality feeds. The expansion of SSF technology might offer scope for the preparation of high-quality feeds through bioconversion and the value addition of low-cost feed ingredients (Esakkiraj et al., 2009). In this context, the major aim of the presently reported study was the reclamation of plant ingredients into nutritionally enhanced (value-added) products. Therefore, the present study has been undertaken to explore the possibility of utilizing two low-cost, locally available plant feedstuffs i.e., sesame oil cake, as a substrate for SSF, and to evaluate the value addition of the substrates for their subsequent use as aqua-feed ingredients.

Endogenous enzymes in the fish digestive system only help to hydrolyze organic macromolecules into simpler compounds. Hence, supplementation of specific exogenous enzymes could be a remedial measure to address the problem (Cheng and Hardy, 2002; Portz and Liebert, 2004; Debnath et al., 2005). Otherwise, pre-treatment with microbial enzymes produced through the process SSF has been indicated to ameliorate feed utilization through the deactivation of ANFs (Ramachandran and Ray, 2007). The de-oiled sesame oil cake (SOC) contains 38.3% crude protein, 8.5% crude lipid, and 5.44% crude fiber and can be incorporated up to 23-25% of the common carp diet (Rangacharyulu, 2007). Replacing 30% of soybean meal protein with sesame seed cake protein in the fish diet significantly improves the body weight of Nile tilapia, *Oreochromis niloticus* (Abdel-Hakim, 2008).

MATERIALS AND METHODS

The solid-state fermentation of SOC was carried out by using commercially available three different formulations of feed probiotics in powder form namely: - AqualactTM, Rhodomax, and Uni-Nutrich plus which contain extracellular enzymes and/or enzyme-producing fish gut bacteria/yeast. In solid-state fermentations, microbial colonization occurs at or near the surfaces of the solid substrate, or in a few cases the soluble substrate supported on the solid insoluble matrix in the environment of low-moisture contents. Solid-state fermentation can be used to provide low-shear environments for the cultivation of shear-sensitive mycelial organisms like yeasts. Solid-state cultivations can be and have been used for the mass production of mycelia and colonies of bacteria which are rich sources of high-quality proteins (Pandey, 1992; Pandey et al., 2001). Therefore, the process of SSF helps in increasing the crude protein content of plant matter through microbial biomass production (MBP) and reduces plant ANFs like tannin and phytic acid through enzymatic degradation. Prior to SSF, the said plant matter was also treated by a conventional physical method like heat treatment (autoclaving) to partially eliminate ANFs. Afterward, samples of SSF processed (at every 24 hours intervals up to 7 days) plant matter were evaluated for contents of nutrients and anti-nutritional factors.

Preparation of substrate/matrix for SSF

The powdered oil cake was first sieved properly through the 400-micron mesh. Then 5 kg of finely sieved oil cake was mixed thoroughly with 3 liters of tap water i.e., up to 60% moisture content to make a dough. The dough was autoclaved at 121°C for 15 minutes at 15 lb pressure by keeping it in a cylindrical stainless-steel vessel. Then the heat-processed dough was allowed to cool down to a tepid warm stage (approximately to 37°C), transferred to a plastic trash can, and was kept ready for inoculation with 'Microbial suspension'.

Preparation of microbial consortium

Approximately 800 ml of 'aquaguard' filtered tap water was collected in a one-liter capacity glass beaker. Then 25 g of intended probiotics powder and 125 g of jaggery were added to the water (at a rate of 5 g probiotics and 25 g jaggery per kg of prepared substrate respectively). It was mixed properly by stirring with a sterilized glass rod for 5 minutes. Then kept static for another 5 minutes to allow the fibers to settle down. The upper part of 800 ml of supernatant or the microbial suspension was then added to the above-said lukewarm oil cake substrate and mixed thoroughly with a sterilized melamine spatula.

Daily collection of fermented feed sample

At every 24 hours interval (every day by 8 pm), approximately 200 g samples in triplicate were taken out into aluminum casseroles by means of a sterilized melamine spatula and kept in an oven at 60 - 65°C for drying. The samples were dried up to a constant moisture level and then packed in air-tight plastic containers, and brought to the laboratory for proximate nutrients and ANFs analysis.

Optimization of the fermentation process

By following the protocol mentioned above, the sesame oil cake (SOC) was subjected to the SSF process by using separately the three probiotics as microbial inoculums. For the said purpose six fermentation chambers were used. Periodically, in every 24 hours interval samples were collected from each fermentation chamber for 7 days, oven-dried, and analyzed for their proximate nutrients and ANFs composition. In each case, optimization of the fermentation process was adjudged by keeping in view the maximum enrichment of crude protein content and maximum reduction of crude fiber content in the sample.

RESULTS AND DISCUSSION

The results of nutritional quality enhancement through SSF of raw SOC by using three commercially available powder form microbial consortia like-Aqualact, Rhodomax and Uni-Nutrich plus are depicted in Table 1 to Table 3. The ANFs like tannin and phytic acid content in raw sesame oil cake was estimated to be 0.96% and 0.47% respectively. With the progression of the fermentation process with the use of the probiotics, none of these two ANFs were detected in the fermented oil cake samples of raw sesame oil cake after 24 h of fermentation. The initial crude protein and crude fiber content of raw sesame oil cake was estimated to be 26.76% and 21.28% respectively. After 24 h of fermentation with Aqualact, the crude protein content of raw sesame oil cake was increased from the initial value by 12.71% (26.76 to 30.12%). Similarly, the crude fiber content decreased from the initial value by 1.90% (21.28 to 20.88%). But during the subsequent hour of fermentation, the crude protein value registered a further increase in the value with a marginal increase in the percentage up to 96 h, thereafter the percentage increase in the crude protein registers a declining trend up to 168 h of fermentation. Due to the continuation of the fermentation process up to 168 h, the crude fiber values registered an increasing trend from 48 h, by the end of 168 h of the fermentation process, the crude fiber content was increased by 22.98% from that of the initial value of 21.28%. Thus, the crude fiber content in 168 h Aqualact fermented sesame oil cake was 26.17%.

From Table 1, it is evident that, within the first 24 h of the fermentation process with Aqualact, the crude protein content of the sesame oil cake registered an increase of 12.71% and a reduction in crude fiber content by 1.90% from the estimated initial values. But with the progression of the fermentation duration, a percentage increase of the crude protein content registered an increasing trend but of lesser magnitude in comparison to the 24 h fermentation process. After 24 h fermentation, the crude fiber content was gradually increased. Further, up to 96 h of the fermentation process, the percentage increase in the crude protein content was minimal and beyond which the value for crude protein content registered a declining trend with

Table 1. Nutritional quality enhancement of sesame oil cake through 'Aqualact' fermentation

Fermentation duration (h)	Composition of Aqualact fermented sesame oil cake samples				Remarks
	Crude Protein (%)*	% increase (↑) or % decrease (↓)	Crude Fiber (%) *	% increase (↑) or % decrease (↓)	
24	$30.16^{a} \pm 0.12$	12.71 (↑)	$20.88^{a} \pm 0.03$	1.90 (↓)	This stage was chosen for mass production
48	$28.82^{b} \pm 0.04$	7.70 (↑)	$21.84^{b} \pm 0.04$	2.63 (↑)	
72	$28.08^{c} \pm 0.06$	4.93 (↑)	$23.03^{c} \pm 0.04$	8.23 (↑)	
96	$27.36^{d} \pm 0.13$	2.24 (↑)	$24.93^{d} \pm 0.04$	17.15 (↑)	
120	$26.55^{e} \pm 0.16$	0.78 (↓)	$25.66^{e} \pm 0.04$	20.58 (↑)	
144	$24.53^{f} \pm 0.08$	8.33 (↓)	$25.76^{e} \pm 0.04$	21.05 (↑)	
168	$23.95^{g} \pm 0.15$	10.50 (↓)	$26.17^{f} \pm 0.04$	22.98 (↑)	

*Values are means ± SE of three determinations. Mean value with same superscripts in the same. columns are not significantly different (P< 0.05).

a percentage increase in the crude fiber content. Hence, considering the percentage increase in crude protein and reduction in the percentage of crude fiber content in the sesame oil cake, the study suggests adopting for 24 h fermentation process for enhancing the nutritional quality of the raw ingredient. The statistical analysis of crude protein and crude fiber content with respect to the duration of the fermentation process reveals a significant difference between 24 h and the rest of the fermentation process.

Table 2. Nutritional quality enhancement of sesame oil cake through 'Rhodomax' fermentation

Fermentation duration (h)	Composition of Rhodomax fermented sesame oil cake samples			
	Crude Protein (%)*	% increase (↑) or % decrease (↓)	Crude Fiber (%)*	% increase (↑) or % decrease (↓)
24	$31.19^{a} \pm 0.05$	16.55 (↑)	$18.57^{a} \pm 0.04$	12.73 (↓)
48	$27.38^{d} \pm 0.10$	2.32 (↑)	$20.25^{b} \pm 0.04$	4.84 (↓)
72	$26.95^{e,\,f} \pm 0.08$	0.71 (↑)	$22.78^{d} \pm 0.09$	7.05 (↑)
96	$27.81^{c} \pm 0.18$	3.92 (↑)	$21.67^{c} \pm 0.05$	1.83 (↑)
120	$28.32^{b} \pm 0.05$	5.83 (↑)	$18.73^{a} \pm 0.10$	11.98 (↓)
144	$27.28^{d,\,e} \pm 0.14$	1.94 (↑)	$23.36^{e} \pm 0.03$	9.77 (↑)
168	$26.78^{f} \pm 0.16$	0.07 (↑)	$25.07^{f} \pm 0.06$	17.81 (↑)

*Values are means ± SE of three determinations. Mean value with same superscripts in the same. Columns are not significantly different (P< 0.05).

From Table 2, it is apparent that, within the first 24 h of the fermentation process with Rhodomax, the crude protein content of the sesame oil cake registered an increase of 16.55% and a reduction in crude fiber content by 12.73% from the estimated values. But with the progression of the fermentation duration, a percentage increase of the crude protein content registered an increasing trend up to 168 h of fermentation but with a lesser magnitude in comparison to the 24 h fermentation process. Up to 48 h of fermentation, the percentage of crude fiber content was gradually decreased. Beyond that, it exhibited an increasing trend up to 168 h of fermentation except only at the stage of 120 h of fermentation where the percentage of crude fiber content decreased by 11.98%. Hence, considering the percentage increase in crude protein and reduction in the percentage of crude fiber in the sesame oil cake, the study suggests adopting for 24 h fermentation process for enhancing the nutritional quality of the raw ingredient.

The statistical analysis of crude protein and crude fiber content with respect to the duration of the fermentation process reveals that after 24 hours of 'Rhodomax' fermentation of sesame oil cake, the crude protein content was increased to its maximum from 26.76% (initial value) to 31.19%, which is significantly different from others and the crude fiber content was decreased from 21.28% to 18.57% which is significantly different from others except only at the stage of 120 h of fermentation where crude fiber content decreased from 21.28% to 18.73%. Similarly, there are no significant differences between the increase in crude protein content in sesame oil cake fermented for 48 h and 144 h, 72 h, and 144 h, and 72 h and 168 h respectively.

Table 3. Nutritional quality enhancement of sesame oil cake through 'Uni-Nutrich plus' fermentation

Fermentation duration (Hours)	Composition of Nutrich plus fermented sesame oil cake samples				Remarks
	Crude Protein (%)*	% increase (↑) or % decrease (↓)	Crude Fiber (%)*	% increase (↑) or % decrease (↓)	
24	$30.71^{a} \pm 0.17$	14.76 (↑)	$20.78^{a} \pm 0.08$	2.35 (↓)	This stage was chosen for mass production
48	$28.74^{b} \pm 0.03$	7.40 (↑)	$22.87^{c} \pm 0.10$	7.47 (↑)	
72	$27.03^{f} \pm 0.08$	1.01 (↑)	$24.76^{d} \pm 0.06$	16.35 (↑)	
96	$27.77^{d, e} \pm 0.05$	3.77 (↑)	$25.49^{e} \pm 0.08$	19.78 (↑)	
120	$28.11^{c} \pm 0.10$	5.04 (↑)	$26.21^{g} \pm 0.10$	23.17 (↑)	
144	$27.86^{c, d} \pm 0.10$	4.11 (↑)	$25.78^{f} \pm 0.08$	21.15 (↑)	
168	$27.56^{e} \pm 0.03$	2.99 (↑)	$22.23^{b} \pm 0.07$	4.46 (↑)	

*Values are means ± SE of three determinations. Mean value with same superscripts in the same column are not significantly different ($P< 0.05$).

From Table 3, it is evident that, within first 24 h of fermentation process with Uni-Nutrich plus, the crude protein content of the sesame oil cake registered an increase by 14.76% and reduction in crude fiber content by 2.35% from the initial estimated values. But with progression of the fermentation duration, percentage increase of the crude protein content registered an increasing trend up to 168 h of fermentation but with a lesser magnitude in comparison to 24 h fermentation process. Within first 24 h of fermentation, the percentage of crude fiber content was decreased. Beyond that, it exhibited an increasing trend up to 168 h of fermentation, recorded highest at the stage of 120 h of fermentation where the percentage of crude fiber content increased by 23.17%.

The statistical analysis of crude protein and crude fiber content with respect to the duration of the fermentation process reveals that just at 24 hours of 'Uni-Nutrich plus' fermentation of sesame oil cake, the crude protein content was increased to its maximum from 26.76% to 30.71%, which is significantly different from others and the crude fiber content was decreased from 21.28% to 20.78% which is significantly different from others. Similarly, there was no significant differences between increases in crude protein content in sesame oil cake fermented for 96 h and 144 h, 96 h and 168 h, and 120 h and 144 h respectively. Maximum nutrient enhancement was recorded at the initial 24 h fermentation stage.

Adoption of some processing techniques like SSF might contribute to enhancing the nutrient value of plant ingredients. Fermentation is a simple and cheap process where there may be an increase in the nutrient level through microbial synthesis (Wee, 1991). In the present study, increased levels of crude protein and decreased levels of crude fiber content were achieved in fermented plant matter in comparison to the raw substrate is consistent with the findings of Bairagi et al. (2002), Ramachandran et al. (2005) and Saha and Ray (2011).

Dynamics of crude protein content in substrates

During fermentative bioprocessing, sometimes the crude protein content of substrates declines. It is caused by a mixture of two or more microbes that are on the dynamics of the different growth rates. Some microbes are growing and multiplying faster, followed by the other and then another, and so on. Some microbes utilize proteins that are formed by the other group of microbes for growth. So sometimes the addition of fermentation time causes declining in the crude protein content of the fermented substrate. Therefore, mechanical fermentation should be done through the utilization of the biochemical properties of a particular microbe or a consortium of microbes.

The increased crude protein in substrates which occurs during the process of fermentation is caused by the work of the microbes and the addition of protein donated from the cell growth of microbes (single-cell proteins). The increase in crude protein content of fermented substrates is also caused due to microbes' use of food ingredients such as carbohydrates and fat for their growth and multiplication. A yeast/fungus/bacteria can synthesize a protein by taking carbon sources of carbohydrates (glucose, sucrose, and maltose from jaggery or molasses), a nitrogen source of inorganic or organic material, and minerals from its substrate. Also, during the incubation period, foods derived from the substrate are used for the growth of microbes. The more the growth of microbes envisages the more microbial biomass production causes more protein in the substrate (Setiyatwan et al., 2018). The protein content increases in the substrate resulting in a proportionate reduction of the carbohydrate content in the substrate. The longer fermentation duration facilitates an increasing chance of bacteria colonies to grow and ultimately the protein, amino acids, and nitrogen content in the substrate will be increased.

Dynamics of crude fiber content in substrates

The inoculum dosage of microbes to the oil cakes significantly affects the crude fiber content of the substrate to be fermented. The higher dosage of inoculum, the higher the crude fiber content of the substrate will be. It is caused by the mycelial growth of yeast. The yeast eats the mycelia wall and contains a lot of cellulose and chitin substances (compounds that have the same function as the plant cell wall). The cellulolytic enzymes thus produced by the yeast is not optimal because of the slow growth of the fungus, so the addition of fermentation time does not affect the crude fiber content in the substrate (Kurniati et al., 2016). Mycelia is a collection of hyphae, hyphae surrounded by a cell wall composed of polysaccharides, and the highest content of cell wall in most fungi is cellulose. The high content of crude fiber in the fermented substrate is due to the crude fiber content of the fungi mycelia.

In contrast, some *Bacillus* group of bacteria, which have the cellulose-degrading ability when inoculated to the fermented substrate secrete cellulolytic enzymes and break down cellulose, and hemicelluloses to simpler forms of carbohydrates (glucose and cellobiose). That is why with the advancement of the fermentation process, the crude fiber content in the substrate decreases.

CONCLUSION

Solid-state fermentation is a technique through which anti-nutritional factors like tannin and phytic acid can be eliminated completely from plant matter and can increase the bioavailability of crude protein, and minerals and effectively reduce crude fiber contents in the sesame oil cake substrate. Due to the reduction in crude fiber content and elimination of anti-nutritional factors, the fermented oil cakes were easily digested and assimilated into the body of fish.

Solid-state fermentation processed sesame oil cake has the potential for application as an alternate protein source in formulated diets for carps.

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