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Effects of Fermentation on Nutritional Quality of *Prosopis Juliflora* Pods as Alternative Fish Feed

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Abstract

Prosopis juliflora pods are extensively used as animal feed source in arid and semiarid regions of the world. The present paper establishes the improvement in the nutritional quality of pods by solid state fermentation using Bacillus subtilis (BS), Bacillus circulans (BC) and Saccharomyces cerevisiae (SC). The pH has decreased in fermented pods. The crude protein has increased in pods fermented with BS (39.33%, 48 h), BC (4.29%, 24 h) and SC (27.29%, 72 h) in comparison to control. Maximum increase (P<0.05) in total soluble sugar (28.66%) and in reducing sugar (49.69%) were observed in Bacillus fermented group. The fermentation resulted in maximum decrease in total fibre (up to 30%) and in hemicelluloses (up to 17. 67%). A significant decrease (P<0.05) in tannin and in phytic acid was observed in pods fermented with all the three microbial cultures; and the level of these antinutrients in fermented pods are found to be in permissible limit for carp. Thus fermented PJ pods emerge as a suitable agrobased candidate for supplementary fish feed..

Keywords *Prosopis juliflora*, fermentation, Antinutritional factors, Non starch polysaccharide.

Introduction

The success of intensive fish culture practice depends on use of nutritionally balanced supplementary feed which accounts for 40 to 60% of total cost of culture management[1]. Formulation of cost effective fish feed using locally available, economical and nutritionally adequate agro products can significantly benefit fish culture. For carp culture, traditionally a mixture of rice bran and various oil cakes are used as supplementary feed[2,3]. Most of the research has been concentrated on the evaluation of legume seeds, oil seeds, leaf meals and their by-products as feed for carp[2,4,5]. In many cases, low protein content, amino acid imbalance and presence of antinutritional factors limit use of agro products directly in formulation of fish feed[6]. This has led to the applications of processing technologies for removal of antinutritional factors and improvement in the nutritional quality of feed to some extent[7]. The improvement of nutritional quality of feed by different processing techniques such as hydrothermical processing[2,8], use of exogenous enzymes[9-11] and fermentation[12,13] have been reported for several plant based supplementary fish feed.

Prosopis juliflora (PJ) (mesquite), family *Fabaceae* is a legume that grows fast in arid and semi-arid regions. It is resistant to heat, drought and salinity. The Prosopis fruit pods have been reported as dietary source for livestock with promising results[14,15] and also used as food source for human in some parts of the world[16]. The pods are rich in carbohydrates (46-52%); and the main soluble sugar present is sucrose[17]. The pods and seeds contain 10–18% and 35% protein respectively with lysine as a predominant amino acid[18,8]. The pods are rich in minerals, Ca, P, Mg, K, Na, Cu, Zn, Fe and Mn as well as in vitamins[17]. However the fibre content of pods is high (26-32 %); they contain antinutritional factors (ANFs) such as tannins, phenolic compounds, phytic acid and trypsin inhibitor; and also contain galactomannan as a major non-starch polysaccharide (NSPs)[19,16]. Thus improvement of the nutritional quality and removal of antinutritional factors in PJ pods (PJP) are important for their effective use as feed in animal diet, especially as a dietary source for fishes.

Fermentation is considered to be a simple, cheap and important processing technique to improve the nutritional quality and to reduce the ANFs from the agro-based fish feed sources[12]. The microbial strains like *Lactobacillus* sp., *Bacillus* sp. and *Saccharomyces cerevisiae* are known to be used in fermentation of feed to improve their nutritional quality[5,12,20]. The *Bacillus* sp. are reported to produce amylolytic, cellulolytic, lipolytic and proteolytic activities during fermentation of agro-material for fish feed[5,12]. It is known that *S. cerevisiae*; a cheap and non-pathogenic saprophytic facultative anaerobe could be used for enhancing the nutritional potential of plant based material by increasing nutrient content and by decreasing ANFs up to some extent[21].

To the best of our knowledge, fermentation of PJ pods with presently selected microbial strains has never been reported. The objective of this work is to investigate the improvement in the nutritional quality of pods and removal of antinutritional factors from pods by fermentation using *Bacillus subtilis*, *Bacillus circulans* and *Saccharomyces cerevisiae* for its intended use as alternative supplementary feed for carps.

Material and Methods

Sample preparation: Dry ripen *Prosopis juliflora* (PJ) pods were obtained from local market, dried at 60°C in hot air oven, ground to fine powder and stored at -20°C.

Microbial strains: The pure cultures of *Bacillus subtilis* (NCIM 2479) (BS), *Bacillus circulans* (NCIM 5049) (BC) and *Saccharomyces cerevisiae* (NCIM 3494) (SC) were procured from National Collection of Industrial Microorganisms (NCIM), Pune. The BS and BC were maintained on nutrient agar slants; and SC was maintained on MGYP (Malt extract Glucose Yeast extract Peptone) agar slant at 4°C.

Preparation of microbial inoculums: The inocula of each microbial strain was prepared by inoculating the cultures into 100 ml of sterile nutrient broth and MGYP broth for *Bacillus* and *Saccharomyces* respectively; and then incubated at 37°C for 24 h on a rotary shaker (120 rpm). The cell density of inoculum was adjusted to 10^8 cfu ml^{-1} by appropriate dilution with sterile distilled water.

Fermentation of PJ Pods: The pods were fermented by solid state fermentation, separately in triplicate for each of the microbial strains in 500 ml Erlenmeyer flask. A mixture of 100 g of finely powdered PJP and distilled water (1:1 W/V) were autoclaved at 121°C for 15 min; and this mixture was inoculated separately with different microbial strains (10^8 cells g^{-1}). Fermentation was carried out in BOD incubator at 37°C up to 96 h with regular collection of samples at an interval of 24 h and immediately their pH was checked (before drying). Fermented samples were dried at 40°C in hot air oven and stored at -20°C for further analysis.

Proximate analysis: The proximate composition of test samples were analysed as per the methods of Association of Official Analytical Chemists[22] as follows: Moisture by drying at 105°C for 24h, protein (N × 6.25) by Micro Kjeldahl, the ash content by ignition at 550°C in a Muffle furnace to constant weight, lipid by using chloroform methanol mixture (10:20), total soluble sugar by phenol sulphuric acid method and the reducing sugar content was analysed by Nelson Somogyi method.

Determination of non-starch polysaccharides: Crude fibre was analysed using Sigma kit (Catalog no. TDF-100A; Sigma, USA). Non starch polysaccharides in terms of acid detergent fibre (ADF), neutral detergent fibre (NDF) and hemicelluloses were estimated using gravimetric method[23].

Analysis of anti-nutritional factors (ANFs): The ANFs total phenol, tannin, phytic acid and saponin were estimated by spectrophotometric methods[24-27].

Statistical analysis: The triplicate data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Tukeys multiple range test ($P < 0.05$) using the SPSS Version 16. Values are expressed as means ± SE.

Results and discussion

The solid state fermentation of PJ pods with *B. subtilis* (BS), *B. circulans* (BC) and *S. cerevisiae* (SC) has resulted into improvement in its nutritional quality by enhancing the major nutrient levels and by decreasing the levels of antinutritional factors as described below.

Table-1
pH and Proximate composition of unfermented and fermented *Prosopis juliflora* pods[1,2]

Fermentation time (h)	pH	Dry matter (%)	Crude protein (%)	Lipid (%)	Ash (%)	Total soluble sugar (%)	Reducing sugar (%)
BS							
Native PJP	4.54± .014^{d}	94.30±.416^{ab}	8.39±.005^{c}	4.48±.070^{a}	7.56±.120^{a}	46.09±.200^{d}	7.11±.055^{a}
24	4.51 ±.001^{cd}	95.13±.033^{bc}	7.73±.020^{b}	4.86±.033^{ab}	6.02±.991^{a}	59.30±.280^{e}	8.18±.034^{c}
48	4.32± .013^{bc}	94.70±.057^{bc}	11.69±.019^{e}	5.43±.176^{ab}	8.79±.043^{a}	21.93±.200^{a}	7.89±.043^{b}
72	4.16 ±.095^{ab}	95.46±.218^{c}	9.62±.004^{d}	4.86±.033^{ab}	8.56±.123^{a}	24.00±.160^{b}	9.46±.050^{e}
96	4.12± .010^{a}	93.40±.028^{a}	4.90±.017^{a}	4.53±.218^{a}	8.51±.203^{a}	42.47±.325^{c}	8.73±.105^{d}
BC							
Native PJP	4.54± .014^{b}	94.30±.416^{a}	8.39±.005^{d}	4.48±.070^{a}	7.56±.120^{a}	46.09±.200^{b}	7.11±.055^{bc}
24	4.43±.005^{b}	94.80±.057^{ab}	8.75±.028^{e}	5.70±.416^{b}	8.50±.058^{b}	32.23±.463^{a}	7.05±.034^{b}
48	4.44±.027^{b}	94.90±.057^{ab}	7.54±.030^{a}	6.76±.088^{c}	8.48±.016^{b}	55.16±.397^{c}	6.54±.044^{a}
72	4.45±.024^{b}	95.10±.057^{ab}	7.85±.013^{b}	6.60±.028^{c}	8.41±.005^{b}	46.99±.293^{b}	10.65±.106^{e}
96	4.20±.003^{a}	95.20±.057^{b}	8.03±.047^{c}	7.21±.109^{c}	8.46±.036^{b}	55.02±.488^{c}	8.24±.060^{d}
SC							
Native PJP	4.54 ±.014^{c}	94.30±.416^{c}	8.39±.005^{b}	4.48±.070^{a}	7.56±.120^{a}	46.09±.200^{d}	7.11±.055^{c}
24	4.24±.017^{b}	91.10±.296^{b}	7.07±.036^{a}	7.05±.014^{c}	7.50±.055^{a}	48.11±.081^{e}	7.46±.049^{c}
48	4.20±.003^{ab}	92.09±.063^{b}	7.35±.173^{a}	6.76±.088^{b}	8.09±.060^{b}	39.32±.124^{b}	4.40±.109^{a}
72	4.13±.025^{a}	91.53±.268^{b}	10.68±.007^{d}	7.50±.057^{d}	8.01±.005^{b}	42.66±.047^{c}	5.12±.014^{b}
96	4.07±.013^{a}	92.22±.097^{b}	10.20±.029^{c}	7.50±.057^{d}	7.49±.026^{a}	33.64±.124^{a}	4.31±.116^{a}

[1]Data are mean values ± SE. [2] Means not sharing a common letter in a column are significantly different at P<0.05 among fermentation period within each group (i.e. native PJP and fermented with BS, BC and SC)

pH, dry matter, ash and lipid content: The effect of fermentation on pH, dry matter, ash and lipid content in pods is shown in table 1. Upon 96 h fermentation of pods with BS, BC and SC, the pH dropped significantly ($P<0.05$) from the initial value of native pods 4.54 to 4.12, 4.20 and 4.07 respectively. A similar reduction in pH of sorghum and soybean meal has been reported by Ibrahim et al. (2005)[28] and Song et al. (2008)[29] during natural fermentation and in during fermentation with *S. cerevisiae* respectively. The decrease in pH during fermentation by all the three cultures could be due to the sugar fermentation with acid production. The negligible difference in dry matter content was found to be significant at $p<0.05$ suggesting transformation of substrates to simpler forms or protein turnover along with evolution of volatile compounds and respiratory gases which may account for the decrease in dry biomass values. The profile of ash content during fermentation also reflects conversion of substrates from one form to another without significant loss in the form of volatile compounds. The increase in lipid content was observed in pods fermented with BC (5.7 to 7.2%) and SC (6.7 to 7.5 %) in comparison with native pods (4.48%). The increase in lipid in fermented pods may be attributed to microbial transformation of carbohydrate to lipid[30,12]. The *S. cerevisiae* has been reported to improve the lipid level of cassava during fermentation[21]. The fermentation of leaf meal and grass pea seed meal with *Bacillus* sp. has been reported to increase the lipid level, especially free fatty acids by Bairagi et al. (2004)[5] and Ramachandran et al. (2005)[12].

Protein contents: The effect of fermentation with BS, BC and SC for different time intervals on the crude protein (CP) content of PJ pods is shown in Table 1. The CP content of native pods is found to be 8.40%; and this is in agreement with protein content of pods reported by Silva et al. (1990)[18]. When pods were fermented with BS, the CP was found to increase upon 48 hrs of fermentation by 1.4 fold which may be attributed to synthesis of microbial enzymes for decomposition of substrates. Subsequently after 48 hrs, the CP content was found to decrease significantly to 4.9% during next 48 hrs of fermentation. This decrease in CP may be due to utilization of pod protein as carbon source for growth of BS cells during fermentation. This is analogous to increase in CP content in soybean meal[31], leaf meal[5] and grass pea seed meal[12] reported elsewhere. The significant increase in protein in pods suggests the role of bio-modification involving proteases and synthesis of protein[12]. The clear changes in CP content of pods were not detected when fermented with BC for different time intervals. The fermentation of pods with SC also resulted in increase ($P< 0.05$) in CP value (10.68%). This could be due to increase in yeast biomass and secretion of extracellular enzymes by yeast as suggested by Oboh and Akindahunsi (2003)[21] and Zhang et al. (2007)[32]. Our findings are in agreement with increase in protein content of maize stalk, cassava and cottonseed meal during fermentation with *S. cerevisiae*[20,21,32]. The increase in protein content of fermented pods, especially with BS in 48 h fermented group, is advantageous for its use in fish feed because dietary protein is an important aspect in increasing fish growth and production.

Soluble sugars: The mesquite pods are known to be rich in soluble sugars (40 to 52%); and the main soluble sugar present is sucrose[16]. In the present study, the values for total soluble sugar and reducing sugar contents in PJ pods were found to be 46.09% and 7.12% respectively (Table 1). The total soluble sugar of pods increased significantly ($P<0.05$) by 28.66% during first 24 h; and then decreased during next 48 hrs of fermentation with BS. Fermentation with BS also resulted in significant increase (33.05%) of reducing sugar during 72 h of fermentation. The total soluble sugar content was found to increase to 55.02% upon 96 h of fermentation of PJ pods by BC. Even the reducing sugar concentration was found to increase during fermentation of PJ pods by BC with maximum value (10.64%) observed at 72 h of fermentation, which then reduced to 8.2% upon subsequent 24 h fermentation (Table 1). The significant increase in total soluble sugar and reducing sugar in pods during fermentation with *Bacillus* Sp. indicates the role of cellulolytic and amylolytic activities in converting complex carbohydrates to soluble sugars as suggested by Yuan et al. (2005)[33] and Ramachandran et al. (2005)[12]. A decline in total soluble sugar in pods during fermentation with BS (48 h to 96 h), BC (24 h) and SC (48 h to 96 h), and a reduction in reducing sugar in pods with SC (up to 96 h) during fermentation indicate utilization of the same reducing sugars as carbon and energy source by the fermenting microorganisms for their growth. An analogous reduction in carbohydrate contents of Jatropha curcas seed cake has been reported during the fermentation with *Bacillus* sp.[34]. Oboh and Akindahunsi (2003)[21] have also reported decrease in carbohydrate in cassava fermented with *S. cerevisiae*.

The crude fibres and non starch polysaccharides (Table 2): The values for crude fibre and other non-starch polysaccharide content of native PJ pods have been found as crude fibre (24.64 %), NDF (47.22%), ADF (32.10%) and hemicelluloses (15.12%) (Table2). During fermentation of PJ pods by all the three microorganisms, a similar profile of crude fibre content was observed (Table 2); that is, the crude fibre content was found to decrease during initial course of fermentation followed by increase during rest of fermentation. There were no incredible changes observed in NDF and ADF content during fermentation of pods. An irregular change in crude fibre content has been observed during the fermentation; an initial decrease during early fermentation followed by increase with increase in fermentation period. The decrease in crude fibre during early fermentation period could be due to solubilization of fibre; whereas increase in later period could be due to accumulation of bacterial cell walls. Pods are known to contain quite high level of fibres and other NSP, mainly galactomannan. The dietary fibres of pod represents 16% to 30% of the pulp mainly

insoluble; and the predominant NSPs found in the pods are cellulose, hemicelluloses mainly galactomannan, lignin, and several others[35,36]. The decrease in hemicelluloses in pods during the fermentation with BS and SC indicates the role played by xylanase and other cellulolytic enzyme activities as reported by Yuan et al. (2005)[33]. The fermentation with *Bacillus* sp. is reported to degrade crude fibres, cellulose and hemicelluloses level in *Leucaena leucocephala* leaf meal, wheat bran and grass pea seed meal[5,33,12]. Presently observed some decrease in fibre as well as NSP content of pods during fermentation with Bacillus suggests the improvement in nutritional quality of fermented pods in comparison to their native form. The nutritionally improved fermented pods can be used in fish diet, as fibres and NSP are known to inhibit nutrient utilization and digestion, and finally affect the health of the fishes[36].

Antinutritional factors (Figure 1-4): The pods are found to contain antinutritional factors saponin (317 mg 100 g^{-1}), tannin (860 mg 100 g^{-1}), phytic acid (181 mg 100 g^{-1}) and total phenol (640 mg 100 g^{-1}). The effect of fermentation on antinutritional factors has been shown in Figure 1-4. A significant decrease ($P<0.05$) in tannin and phytic acid has been observed in all the fermented groups with BS, BC and SC; and phytic acid level was reduced to below detectable level in pods fermented for 96 h with *Bacillus* sp. The tannin content of pods was found to have reduced by 16% (96 hrs), 15% (96 hrs) and 14% (72 hrs) when fermented with BS, BC and SC respectively. The level of saponin in the pods was reduced by 11% (BS) and 49% (BC) during 72 h fermentation; however it was found to be higher in 24 h and 48 h fermented groups in comparison to native pods. The fermentation of pods with SC resulted in increase ($P<0.05$) in saponin in all the fermented groups except 24 h fermented group. The total phenol content of pods increased continuously up to 72 h fermentation with BS and then decreased. Fermentation of pods with BC and SC resulted in transient decrease (by 15.21% to 18.69%) in total phenol content during initial fermentation, which subsequent increase to the same level as in native pods. The fermentation of *Leucaena leucocephala* and *Lathyrus sativus* meal with *Bacillus* sp. is known to reduce tannin and phytic acid[5,12]. The extra cellular amylolytic, cellulolytic and proteolytic activities of presently used microbial strains could be responsible for reduction in tannin and phytic acid contents of fermented pods[37]. The reduction in phytic acid could be due to activity of phytase produced by fermenting organisms as reported by Kerovuo et al. (2000)[38]. The lowering of pH during fermentation might have played a role in maintaining phytase activity[39]. The exact reason for initial increase and then decrease in saponin content of pods during fermentation has not been understood clearly. The presently observed increase in total phenol content of pods during fermentation with BS is in agreement with the increase in polyphenol content of fermented products[40]. The plant based feed are known to contain anti-nutrients and they have a detrimental effect on the growth of the fishes mainly by inhibiting the nutrient digestion. Common carp has been shown to tolerate 2% tannin without any adverse effect on the growth[41].

Table-2
Fibre fractions composition of unfermented and fermented *Prosopis juliflora* pods on % dry matter basis[1, 2]

Microbial strain	Fermentation period (h)	Crude fibre (%)	NDF (%)	ADF (%)	Hemi-cellulose (%)
BS					
	Native PJP	24.64±.166^{d}	47.22±.003^{a}	32.10±.057^{a}	15.12±.060^{d}
	24	24.67±.133^{d}	47.17±.095^{a}	33.70±.091^{c}	13.47±.178^{b}
	48	17.37±.067^{a}	47.17±.088^{a}	33.06±.033^{b}	14.10±.057^{bc}
	72	21.81±.003^{b}	48.08±.047^{b}	33.16±.088^{b}	14.91±.118^{d}
	96	23.71±.100^{c}	47.18±.096^{a}	34.73±.120^{d}	12.45±.194^{a}
BC					
	Native PJP	24.64±.166^{e}	47.21±.003^{c}	32.10±.057^{b}	15.12±.060^{b}
	24	17.71±.100^{a}	47.00±.007^{b}	33.66±.133^{c}	13.33±.133^{a}
	48	18.81±.003^{b}	43.18±.036^{a}	30.10±.100^{a}	13.08±.079^{a}
	72	22.80±.005^{d}	48.06±.056^{d}	32.20±.057^{b}	15.85±.029^{c}
	96	21.30±.005^{c}	49.17±.033^{e}	33.60±.057^{c}	15.56±.033^{c}
SC					
	Native PJP	24.64±.166^{d}	47.21±.003^{b}	32.10±.057^{b}	15.12±.060^{b}
	24	17.30±.008^{a}	49.15±.028^{d}	33.60±.115^{c}	15.55±.132^{b}
	48	22.80±.005^{b}	47.70±.057^{c}	30.16±.088^{a}	17.53±.120^{c}
	72	23.30±.003^{c}	47.82±.025^{c}	30.16±.087^{a}	17.65±.112^{c}
	96	23.30±.003^{c}	43.10±.057^{a}	30.10±.103^{a}	13.00±.152^{a}

[1]Data are mean values ± SE. [2] Means not sharing a common letter in a column are significantly different at $P<0.05$ among fermentation period within each group (i.e. native PJP and fermented with BS, BC and SC).

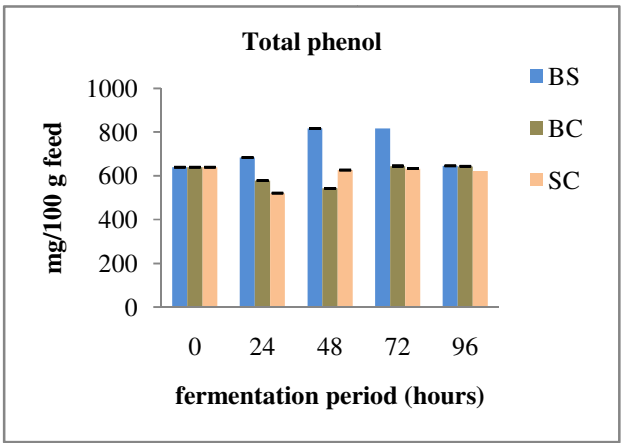

Figure-1

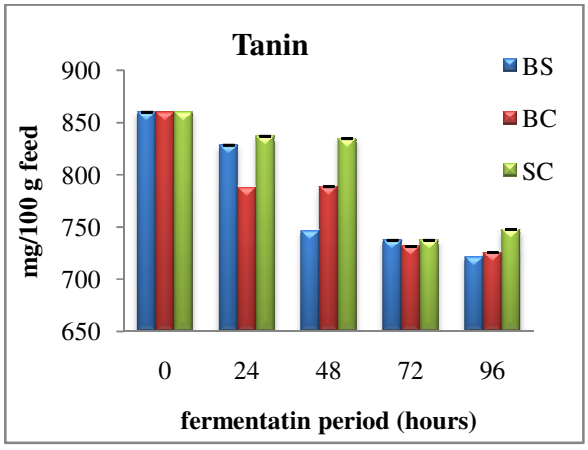

Figure-2

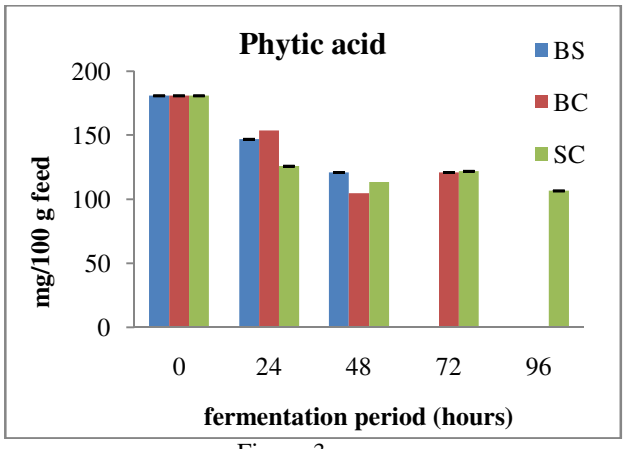

Figure-3

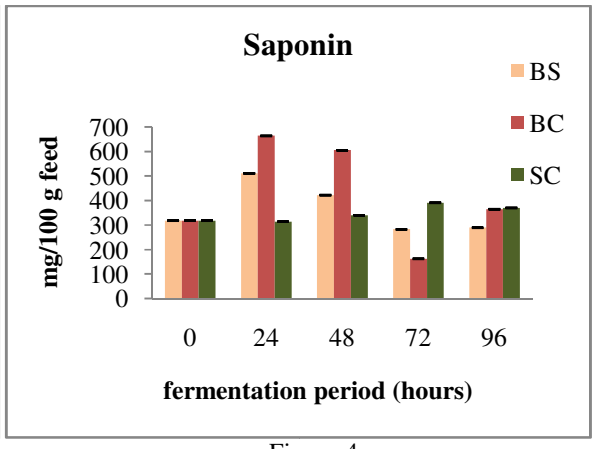

Figure-4

Figure 1-4
Level of some Anti-nutritional factors in unfermented and fermented *Prosopis juliflora* pods (mg 100 g^{-1} of dry matter)

Conclusion

The present results indicate that the nutritional quality of pods is improved by solid state fermentation. The *Bacillus* sp. is found to be more effective in improving the nutritional quality of pods with respect to increasing the levels of protein and soluble sugars content, decreasing hemicellulose content and reducing the level of phytic acid as well as tannin. A significant decrease in tannin and complete elimination of phytic acid in fermented pods suggest the feasibility of use of fermented pods as dietary source for fishes. The findings suggest that the fermentation of pods using a combination of *Bacillus* and *Saccharomyces* needs investigation for the further improvement in nutritional quality of pods in fish feed.

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