Research article

***Lactobacillus plantarum* fermentation to reduce anti-nutritional contents in peanut, mustard and sesame**Sandya B. K¹., Gowthami G. A¹., Harish Nayaka M. A²., Gunashree B. S.¹¹Department of Studies and Research in Microbiology, Mangalore University, Jnana Kaveri Post Graduate Centre, Chikka Aluvara, Kodagu 571 232, Karnataka, India²Department of Studies in Sugar Technology, Sir M. Visvesvaraya Post Graduate Centre, University of Mysore, Tubinakere, Mandya, Karnataka, India

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Corresponding author: **Gunashree B. Shivanna**. Email: gunashree_bs@yahoo.co.in**ABSTRACT**

Introduction and Aim: Oilseeds are an important source of nutrition in developing countries, which are either consumed directly or as snacks. However, the presence of anti-nutritional factors limits their use. This study evaluated the potential application of probiotic *Lactobacillus plantarum* in reducing the anti-nutritional content in peanut, mustard and sesame.

Materials and Methods: Peanuts, sesame and mustard seeds were coarsely ground, defatted by Soxhlet extraction method using hexane and fermented by submerged fermentation method using *Lactobacillus plantarum*. After fermentation for 72hrs, the contents were centrifuged and the pellets in addition to defatted raw sample were analyzed for anti-nutrients like polyphenols, oxalates, trypsin inhibitors and lectins using standard protocols.

Results: Polyphenol content was reduced by 26.40, 46.70 and 41.50%, while oxalate content exhibited 61.50, 32.70 and 37.70% reduction in peanut, mustard and sesame respectively. Trypsin inhibitor activity was reduced by 80.00, 12.13 and 77.78%, while lectin exhibited 87.50, 62.50 and 64.87% reduction in peanut, mustard and sesame respectively.

Conclusion: Peanut showed maximum reduction in oxalates, trypsin inhibitors and lectins followed by sesame and mustard. However, highest polyphenol reduction was exhibited in sesame. Hence, from the present investigation, it is found that fermentation may be a promising tool in reducing anti-nutritional factors from oil seeds.

Keywords: *Lactobacillus plantarum*; anti-nutrients; submerged fermentation.

INTRODUCTION

Lactic acid bacteria (LAB) are a heterogeneous group of bacteria which falls under GRAS (generally regarded as safe) category of food additives. They are being used since time immemorial for fermentation of foods due to their ability to produce flavours, aroma and texture and preservation of food. LAB have variety of uses in current food systems ranging from fermented dairy products, fermented vegetables, alcoholic beverages, fermented dough, fermented meat products, probiotics, etc. *Lactobacillus plantarum* is one of the most versatile probiotics present and has properties ranging from production of flavours and aroma to reduction of anti-nutrients. Its use is safe and the method of use is also simple which makes it the organism of choice. It produces enzymes that can degrade the anti-nutrients (1- 5).

Oilseeds, which form a large part of diets in developing and underdeveloped countries, are rich in proteins and fats. They are either consumed directly as snacks or added in other foods as condiments. They however contain anti-nutrients which lower their nutritional quality and hinder their bioavailability or digestion of existing nutrients. They can also produce off-flavours and off-tastes in foods

and some also have toxic effects on human health. Oilseeds contain anti-nutrients such as tannins, polyphenols, trypsin inhibitors, oxalates, lectins, metal chelators and cyanogens (5). Their presence is a major factor for lesser acceptability of oilseeds within the daily diet. Their reduction is of importance as it can increase their consumption and make their use much wider.

Polyphenols have the ability to bind to iron in the food and make it unavailable for absorption. They can also inhibit absorption of non-haem dietary iron (6). Oxalate, salt of oxalic acid binds with minerals like magnesium or calcium to form their respective insoluble salts. These salts precipitate in the kidneys and can lead to formation of kidney stones (7). Trypsin inhibitors are protease inhibitors that inhibit trypsin activity in the gut and prevent protein digestion. They form indigestible complexes with dietary proteins making them unavailable for digestion (8). Lectins are glycoproteins with non-catalytic binding domain that can bind reversibly to specific oligosaccharides or monosaccharides which hinder their digestion and make them unavailable in the gut. They can also bind to carbohydrate binding receptors and reduce carbohydrate absorption (9).

Fermentation is an ingenious method to reduce the anti-nutritional content of oilseeds. Along with reducing the concentration of anti-nutrients, it also improves flavour, texture and taste of the fermented food and increase the bioavailability and digestibility of the food (10-11). Fermentation with LAB, especially *L. plantarum*, will also make the food being consumed of probiotic nature and the enzymes produced by it can aid in digestion (10). Fermentation with LAB species has been shown to reduce the anti-nutrients content in foods and also improves their sensory quality (11). It is shown in the earlier investigation that *L. plantarum* reduced the anti-nutrients content in food mixture, soybean, sorghum flour, grass pea flour, lebeck seeds and cocoyam tuber (12-14). Fermentation also improves digestibility of proteins, starch, increase bioavailability of minerals, increase fibre content and improve the overall acceptability of the food product (13-16).

The aim of this investigation was to evaluate the scope of *Lactobacillus plantarum* in reducing the anti-nutrient concentration of oilseeds. The oilseeds selected namely, peanut, mustard and sesame are the ones that are most commonly used by the general public in daily life. Probiotic fermentation reduced their anti-nutritional content thereby making them safe for consumption and daily use and also adds to their nutritional value and sensory profile and makes the fermented food a source of probiotics.

MATERIALS AND METHODS

The oilseeds such as peanuts, sesame and mustard were procured from local market at Kushalnagar, Kodagu (India). The culture of *Lactobacillus plantarum* was obtained from Department of Food Microbiology, CSIR-CFTRI, Mysore, Karnataka, INDIA.

Fermentation of seeds

The oilseeds were washed with tap water to remove any dirt and extraneous matter. They were then dried and coarsely ground using a mixer-grinder. The ground oilseeds were defatted using Soxhlet extraction method using petroleum ether and were suspended in Man Rogosa and Sharpe broth at 15% concentration. The broth suspension was inoculated with *Lactobacillus plantarum* culture and incubated at 37°C for 72hrs. in a shaker incubator. The culture broth was centrifuged at 5000 rpm and the pellet containing the oilseeds grits was dried in hot air oven at 50°C to remove moisture and cease fermentation. The dried fermented seeds grits and the supernatant were both used to measure the amount of anti-nutrients.

Estimation of total polyphenols

1g of powdered, defatted and fermented seed sample was extracted by refluxing with 1% hydrochloric

acid-methanol at 60°C for 40 minutes and the extracts were pooled and concentrated under vacuum in a rotary flash evaporator (17). 2.5 ml of Folin-Ciocalteu reagent and 7.5 ml of 15% sodium carbonate were added to 0.1 ml acidified methanolic extract and mixed thoroughly. The volume was made up to 50 ml and the mixture was incubated at room temperature for 30 minutes. The absorbance was recorded at 760 nm and the concentration of polyphenols was estimated by extrapolating the values of sample on a pre-prepared standard curve using Gallic acid. The results were expressed as mg Gallic acid equivalents [GAE/ 100g of dry sample] (18).

Estimation of oxalates

Soluble and insoluble oxalates were extracted with 15 ml distilled water and 2N hydrochloric acid respectively. The sample suspensions were heated in a boiling water bath for 15-20 minutes and cooled down to room temperature. It was filtered through Whatman No. 1 filter paper, washed with distilled water and the volume was made up to 50 ml (19). 0.25 ml bromophenol blue, 0.4 ml potassium dichromate and 1 ml sulphuric acid were added to 1 ml sample and incubated in a boiling water bath for 10 minutes. Reaction was terminated by adding 1 ml sodium hydroxide and absorbance was read at 600 nm. The oxalate concentration was estimated using a standard curve prepared using oxalic acid.

Estimation of trypsin inhibitors

Trypsin inhibitors were extracted using 10 ml 0.025 N hydrochloric acid at 26±5°C with constant stirring. The solution was filtered through Whatman No. 1 filter paper (20). 1 ml sample was mixed with 2 ml trypsin solution and incubated at 37°C for 10 minutes. 5 ml of pre-warmed N- α -benzoyl-D,L-Arginine-p-Nitroanilide (BAPNA) solution was added to it and the contents were stirred in a vortex mixer for 10 min. Reaction was terminated by addition of 1 ml 30% acetic acid and the absorbance was read at 410nm (21). The trypsin inhibitor activity was estimated by using a standard curve prepared for trypsin. One Trypsin Unit (TU) is arbitrarily defined as an increase of 0.01 absorbance at 280 nm in 20 mins per 10 ml of the reaction mixture under set conditions. Trypsin inhibitors activity is defined as the number of trypsin units inhibited (TUI).

Lectin content estimation

1 g sample was mixed with 50 ml 0.15 M NaCl and incubated at room temperature for 6 hours and filtered through Whatman No. 1 filter paper (22). 5 ml alkaline solution and 0.5 ml Folin-Ciocalteu reagents were added to 1 ml sample and incubated at room temperature for 30 minutes. Absorbance was recorded at 670 nm. The lectin concentration was estimated by extrapolating the values of sample on a pre-prepared standard curve for dextran.

RESULTS

Polyphenol content

The results obtained in the present study showed a reduction in polyphenol content of oilseeds during *Lactobacillus plantarum* fermentation. The results are shown in Table 1. Raw peanuts, mustard and

sesame contained 910 ± 1.32 , 881 ± 1.37 and 889 ± 1.31 $\mu\text{g/g}$ of polyphenol content. It was reduced to 670 ± 1.48 , 470 ± 1.31 and 520 ± 1.05 $\mu\text{g/g}$ after fermentation. Lactic acid bacteria have been known to produce β -glucosidase enzyme that degrade polyphenols into bioactive phenolics responsible for antioxidant activity.

Table 1: Polyphenol content in raw and fermented oilseeds ($\mu\text{g/g}$)

Oilseeds	Raw	Fermented	% Reduction
Peanut	910 ± 1.32	670 ± 1.48	26.4
Mustard	881 ± 1.37	470 ± 1.31	46.7
Sesame	889 ± 1.31	520 ± 1.05	41.5

Oxalate content

A reduction in oxalate content of oilseeds was obtained during *L. plantarum* fermentation. Raw peanuts, mustard and sesame showed 780 ± 1.35 , 490 ± 1.21 and 450 ± 1.11 $\mu\text{g/g}$ of oxalate content

respectively. It was reduced to 300 ± 1.06 , 330 ± 1.39 and 280 ± 1.25 $\mu\text{g/g}$ after fermentation (Table 2). Peanut (61.6%) exhibited maximum reduction in oxalate content followed by sesame (32.7%) and mustard (37.8%).

Table 2: Oxalate content in raw and fermented oilseeds ($\mu\text{g/g}$)

Oilseeds	Raw	Fermented	Supernatant	% Reduction
Peanut	780 ± 1.35	300 ± 1.06	425 ± 1.08	61.6
Mustard	490 ± 1.21	330 ± 1.39	203 ± 1.15	32.7
Sesame	450 ± 1.11	280 ± 1.25	214 ± 1.15	37.8

Trypsin inhibitor activity

In the present investigation there was a reduction in trypsin inhibitor activity of tested oilseeds during *L. plantarum* fermentation. Raw peanuts, mustard and sesame contained 400 ± 1.44 , 330 ± 1.15 and 450 ± 1.49 $\mu\text{g/g}$ of trypsin inhibitor activity (Table 3). It was reduced to 80 ± 1.42 , 290 ± 1.20 , and 100 ± 1.23 $\mu\text{g/g}$ after fermentation. Peanut exhibited maximum

reduction of 80% in trypsin inhibitor activity followed by sesame (77.8%) and mustard (12.2%). Trypsin inhibitors are low molecular weight proteins responsible for inhibiting the activity of trypsin, a digestive enzyme. *L. plantarum* produce protease during fermentation that degrades the trypsin inhibitors into smaller subunits or free amino acids and deactivates them.

Table 3: Trypsin inhibitor content in raw and fermented oilseeds ($\mu\text{g/g}$)

Oilseeds	Raw	Fermented	Supernatant	% Reduction
Peanut	400 ± 1.44	80 ± 1.42	112 ± 1.31	80.0
Mustard	330 ± 1.15	290 ± 1.20	110 ± 1.31	12.2
Sesame	450 ± 1.49	100 ± 1.23	198 ± 1.25	77.8

Lectin content

L. plantarum fermentation showed the reduction in lectin content of oilseeds. Raw peanuts, mustard and sesame showed 80 ± 1.35 , 40 ± 1.28 , and 37 ± 1.08 $\mu\text{g/g}$ of lectin content. It was reduced to 10 ± 0.72 , 15

± 0.70 and 13 ± 0.96 $\mu\text{g/g}$ after fermentation. Peanut exhibited maximum reduction in lectin content (87.5%) followed by sesame (64.9%) and mustard (62.5%).

Table 4: Lectin content in raw and fermented oilseeds ($\mu\text{g/g}$)

Oilseeds	Raw	Fermented	Supernatant	% Reduction
Peanut	80 ± 1.35	10 ± 0.72	50 ± 1.35	87.5
Mustard	40 ± 1.28	15 ± 0.70	32 ± 1.42	62.5
Sesame	37 ± 1.08	13 ± 0.96	25 ± 1.15	64.9

DISCUSSION

Rodríguez *et al.*, (1) have reported activity of phenolic acid decarboxylases in *Lactobacillus plantarum* which can decarboxylate polyphenols into their vinyl derivatives and reduce their content in food. *L. plantarum* also produces extracellular tannase which can depolymerize high molecular weight tannins into low molecular weight tannins,

thereby reducing the polyphenol content. Rodríguez *et al.*, (2) also indicated the molecular pathway involved in the process where, tannic acid is hydrolysed to gallic acid and glucose, and gallic acid is further decarboxylated to pyrogallol. It is shown in the earlier report that there was 20 and 19% reduction in polyphenol content of food mixture when fermented with *Lactobacillus plantarum* and

Lactobacillus casei (10). A 93% reduction in tannins in soybean after 5 days of fermentation with *L. plantarum* was also reported earlier (13). The reduction was higher with fermentation than cooking or roasting. The enzymatic activity of *L. plantarum* was proved to be responsible for reduction of antinutritional content. A comparative study was made to find out the efficiency of starter cultures in removing anti-nutrients from ogwo (fermented sorghum- potato gruel). *L. plantarum* was found most efficient out of all the starter cultures used with 56% reduction in polyphenol content. *L. plantarum* proved to be better than *L. fermentum*, *L. acidophilus*, *Saccharomyces cerevisiae*, and *Geosporum candidum* (13). *L. plantarum* was shown to reduce tannin content by 83% in *Phaseolus vulgaris* after 48 hours of fermentation (18). The reduction was more with *L. plantarum* fermentation than with natural fermentation. Similar results were reported (13) in sorghum flour where *L. plantarum* fermentation (96.7%) was more effective than germination (16.12%) in reducing tannin content. However, the present investigation showed 46.7, 41.5 and 26.4% reduction of polyphenols in mustard, sesame and peanuts respectively.

L. plantarum has been proved to produce oxalate decarboxylase, an oxalate degrading enzyme (3). Oxalate is dicarboxylic acid dianion and has two carbon backbone held together by a single covalent bond. The enzyme is involved in catalytic conversion of oxalate into carbon dioxide and formate by cleaving the inert C-C bond (4). Manganese (II) and dioxygen are needed for the catalysis. Pumpkin and sorghum flours when fermented with *L. plantarum* had a 6 times lower oxalate content than their raw forms (12). Olagunju, and Ifesan (11) reported a time dependent decrease in oxalate content of sesame seeds. Oxalate content decreased from 1.05 ± 0.10 to 0.48 ± 0.052 after 96 hours of fermentation with their natural microflora. Adegbehingbe (15) compared the efficacy of starter cultures, viz., *L. fermentum*, *L. acidophilus*, *L. plantarum*, *G. candidum*, *S. cerevisiae*, in removing antinutrients from ogwo (fermented sorghum-potato gruel). Maximum reduction in oxalate content was exhibited by *S. cerevisiae* (76%) followed by *L. acidophilus* (73%). *L. plantarum* degraded 67% oxalate content from ogwo (fermented sorghum-potato gruel) after 48 hours of fermentation. Ojha et al. (2018) compared efficiency of malting and *L. plantarum* fermentation in reducing the oxalate content. Fermentation with *L. plantarum* for 48 hours reduced the oxalate content by 67.85%, which is 18.75% more than germination. Adegbehingbe et al., (16) reported a 68% decrease in the oxalate content of African Breadfruit seeds after it was subjected to mixed fermentation consisting of *B. subtilis*, *B. pumilis*, *S. aureus*, *L. plantarum*, *L. bulgaricus*, *Leuconostoc mesenteroides*, *A. niger*, *A. flavus* and *S. cerevisiae*. Hassan et al., (14) used

mixed fermentation and pure culture fermentation with *A. niger*, *A. fumigatus*, *Rhizopus stolonifer*, *Penicillium chrysogenum*, *Candida albicans*, *Geotricum albidum*, *Bacillus pumilis*, *L. plantarum*, *L. mesenteroides*, *Pediococcus cerevisiae* and *L. brevis* to reduce the antinutrient content of cocoyam tuber (*Colocasia esculenta* L). *L. plantarum* reduced 52% of phytate content whereas maximum reduction was observed after *B. pumilis* fermentation (62%).

A complete removal of trypsin inhibitors (initial concentration of 255.44 ± 4.06) from food mixture when fermented with *L. plantarum* and *L. casei* has been reported (13). Fermentation also improves *in-vitro* digestibility of proteins. It is also reported earlier to have a 98% reduction in trypsin inhibitor activity of soybean after *L. plantarum* fermentation for 5 days. The reduction was higher with fermentation than cooking or roasting. A 12% decrease in the activity of chymotrypsin inhibitors in common bean flour after fermentation with *L. plantarum* for 48hrs has been reported (5). *L. plantarum* was shown to reduce the trypsin inhibitor activity by 57% in *Phaseolus vulgaris* after 48 hours of fermentation (23). Similar results were reported by (24) in reduction of antinutrients of cowpeas using *L. plantarum* fermentation. Fermentation was more effective than cooking and germination, but slightly less effective than pressure cooking and *Rhizopus oligosporus* fermentation in reducing the trypsin inhibitor activity of cowpeas.

Lectin, a stored glycoprotein, is hydrolysed into simpler and available units. The reduction in lectin content can be attributed to degradation of proteins by microbial enzymes into simpler and soluble products (5). Lectin has been reported to act as nutrient substrate for the growth of *L. plantarum* (25). This could be the possible reason for lectin degradation by *L. plantarum*. In the earlier studies a comparison between natural and *L. plantarum* fermentation in reducing the antinutrients of common bean flour has been made (5). The fermentation process reduced 50% lectin content from the flour while fermentation followed by autoclaving removed lectin content completely.

CONCLUSION

Fermentation with *L. plantarum* resulted in significant reduction of anti-nutritional factors in the oilseeds. 26-46% reduction was observed in polyphenol content and 31-61% reduction in oxalate content. Trypsin inhibitors activity was reduced by 12-80% whereas lectin content decreased by 62-87%. Peanut exhibited highest reduction in anti-nutrients such as oxalates, trypsin inhibitors and lectins and least reduction in polyphenols at the end of fermentation, followed by sesame and mustard. However mustard showed highest reduction of 46.7% in polyphenols. Thus fermentation makes the oilseeds safer for human consumption and enhances their

nutritional availability. Further studies can be conducted on scope of reduction of other anti-nutrients like cyanogenic glycosides, phytates, oligosaccharides, etc. Studies can also be focused on other under-utilised oilseeds and grains to increase their use and diversify the diets. Also, other probiotic bacteria and fungi can be explored to reduce the anti-nutritional content of grains and oilseeds.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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