Enhancement of bioavailable iron and calcium contents in fermented linseed (Linum usitatissimum L.) beverages

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ABSTRACT

Linseed (Linum usitatissimum L.) is considered as a nutritious food because of exceptionally high alpha-linolenic acid (ALA) content, dietary fiber, quality protein and phytoestrogens. It is rich in minerals (100 g of seeds contain 350-431 mg of magnesium and 236-250 mg of calcium) and has very low amount of sodium. It also contains anti-nutritional factors, especially phytic acid that interferes with the bioavailability of nutrients like calcium and iron. Fermentation increases the nutritional quality of foods by reducing anti-nutritional factors. Probiotic cultures viz., Lactobacillus acidophilus, Bacillus mesentericus and lactic acid bacterial isolate LAB-3 were used to produce fermented linseed beverage and the quantity of phytic acid, bioavailability of iron and calcium were estimated. Bioavailability of iron and calcium increased by fermentation. The highest bioavailable iron and calcium were observed in L. acidophilus fermentation (4.40 mg and 250.41 mg /100 g seeds, respectively) followed by LAB-3 and Bacillus mesentericus compared to raw seeds that contain 0.89 mg of iron and 125 mg of calcium /100g of seeds. Phytic acid content was high in raw seeds (1392 mg /100 g seeds) and fermentation with L. acidophilus recorded 856 mg phytic acid /100 g seeds resulting in 38.51 % reduction. LAB-3 and B. mesentericus showed approximately 32 % reduction in phytic acid content. The reduction in phytic acid content is significantly high. Fermentation using probiotic bacteria enhanced the bioavailability of iron and calcium by reducing phytic acid. Hence, this study leads to a conclusion that, microbial intervention can be adopted to reduce the anti-nutritional factors and enhance the nutritional quality of linseed.

Key words: Antinutritional factor, Bioavailability, Calcium, Fermentation, Iron, Linseed, Phytic acid.

INTRODUCTION

Linseed (Linum usitatissimum L.) belongs to family Linaceae, seeds contain oil that finds use for edible purpose (Archana et al., 2009). The important linseed growing countries include India, China, United States and Ethiopia. India ranks first in linseed production in terms of acreage (23.8 % of the total) and third in production (10.2 % of the world’s production) (Nivetha et al., 2017). Linseed is mainly cultivated in Madhya Pradesh, Maharashtra, Chhattisgarh and Bihar states of India. Linseed preparations are considered for its nutrients and therapeutical properties, and used in culinaries (Gaida, 2010; Chauhan et al., 2009; Ganorkar and Jain, 2013). Linseed is the richest vegetable source of poly unsaturated fatty acid (PUFA) i.e., alfa-linolenic acid (ALA), an omega-3 fatty acid, phytoestrogens, lignans and dietary fibers. ALA is beneficial for cardiovascular diseases and is known to reduce blood lipids. It contains high quality protein (Ganorkar and Jain, 2013; Bharath et al., 2017). Dietary fibers help to reduce constipation and serve as a hypcholesterolemic agent. Lignans act as antioxidants and have anticancer properties (Rubilar et al., 2010). Linseeds contain anti-nutrients having adverse effects on the health and well-being of human beings e.g., cyanogenic glycosides, phytic acid, tannins and phenolics (Kajla et al., 2014). Several research studies report that microbial fermentations improve nutritional value and reduce anti-nutritional factors thus enhancing bioavailability of minerals and starch (Sood et al., 2000; Mukherjee et al., 2016). Fermented foods are more nutritious than their non-fermented counterparts. These properties make it more favourable for food technologists to explore and develop nutraceutical foods (Giada, 2010). Hence, an attempt was made to develop a fermented beverage of linseed by exploring lactic acid bacteria and using honey as a prebiotic to reduce anti-nutritional factors and to improve the nutritional quality of product.

MATERIALS AND METHODS

Collection of seeds and bacterial cultures: Linseeds were procured from local markets of Bengaluru, Karnataka (India). An efficient phytate degrading isolate LAB-3 has been used in this study, which is previously reported from our laboratory (Nivetha et al., 2017). All the standardized parameters viz., concentrations of substrate, honey, sugar, inoculum, temperature and duration were followed as previously reported by Nivetha et al. (2017). Lactobacillus acidophilus (MTCC-10307) obtained from Microbial Type Culture

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Collection (Chandigarh, India) and Bacillus mesentericus isolated from probiotic sachet were used as inoculants. The bacterial cultures were maintained on de Man, Rogosa and Sharpe (MRS) agar and 48-hours-old cultures were used for the experiment (de Man et al., 1960).

Preparation of linseed beverage: The experiment was designed with the following treatments: T₁ - Raw seed powder, T₂ - Roasted seed powder (RSP), T₃ - RSP + Lactobacillus acidophilus, T₄ - RSP + Bacillus mesentericus and T₅ - RSP + isolate LAB-3.

Estimation of dissociated phytic acid: Phytic acid, dissociated from beverage samples was determined by using Wade’s reagent (Vaintraub and Lapteva, 1988). The sample extract was modified by salt treatment (Gao et al., 2007). Phytic acid degradation due to heat was prevented by drying samples at 47±2°C in an oven for 24 h. Lumps formed during drying were pounded to fine powder. The samples were sieved using 0.45 mm mesh size, packed in airtight containers and stored in refrigerator at 4°C for further analysis.

Phytic acid content was estimated using method as described by Latta and Eskin (1980). Phytates of the samples were extracted using acid and water. HCl (2.4 %) and sterile double distilled water added with cycloheximide and tetracycline were used in acid extraction and water extraction methods, respectively. The acid extracted samples were diluted 25 times and water extracted samples were diluted 10 times. The diluted sample (6 ml) was combined with 2 ml of Wade reagent (0.03 % FeCl₃, 6H₂O + 0.3 sulfosalicylic acid). Contents were thoroughly mixed on a vortex and centrifuged, followed by washing with ammonia and stored in refrigerator at 4°C for further analy.

Standard graph for phytic acid (phytin-P) was plotted using sodium phytate at different concentrations viz., 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00 and 5.00 ml.

Estimation of bioavailable iron: Bioavailable iron was determined from dialysates following standard procedure as described by Rao and Prabhavathi (1978). Briefly, dialysates at pH 7.5 were added with 5 ml of acetate buffer (pH 4.5) to 10 ml aliquot followed by 2 ml of dipyrindyl solution and volume was made to 25 ml with double distilled water, and kept for incubation for 30 min. After incubation, observations were recorded using UV-VIS spectrophotometer (Shimadzu, Japan) at 510 nm.

The bioavailable iron was calculated by comparing the obtained values with standard graph. Standard graph for iron was plotted using ferrous ammonium sulphate at different concentrations viz., 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00 and 5.00 ml.

Estimation of bioavailable calcium: Bioavailable calcium was determined from dialysates following standard procedure of Association of Official Analytical Chemists (Horwitz and Latimer, 2005). The amount of calcium present in the dialysate was precipitated as oxalate and titrated against standard potassium permanganate. Dialysate (3 ml) was taken in a centrifuge tube and 2 ml of double distilled water was added followed by 1.5 ml of 4.0% ammonium oxalate solution. Contents were mixed thoroughly and left for 12 h. Samples were centrifuged for 10 min at 6000 rpm, supernatant was discarded and precipitate was stirred and tubes were washed with 4-6 ml of dilute ammonia (2.0 %) to remove ammonium oxalate. Precipitate obtained was dissolved in 2 ml of 1N sulphuric acid before titration. Tubes were heated in boiling water bath for 1 min and titrated against standard 0.01N KMnO₄ solution to a pink colour. Blank was prepared with water, precipitated with oxalate and centrifuged, followed by washing with ammonia and titrating 2 ml of H₂SO₄ with KMnO₄.

Calcium (mg vol⁻¹ of dialysate) = [(Z - B) x 0.2004 x A] / 2
Z = Amount of 0.01N KMnO₄ required to titrate sample (ml)
B = Amount of 0.01N KMnO₄ required to titrate 2 ml of sulphuric acid, blank (ml)
A = Volume of dialysate (ml)

Statistical analysis: The data obtained from the experiments were subjected to statistical analysis to evaluate treatment effects. Analysis was carried out by completely randomized design using WASP-1 tool. Critical difference values were used to locate significant mean difference.

RESULTS AND DISCUSSION
Phytic acid contents in fermented beverages were determined and are presented in Table 1. The highest phytic acid was present in beverages prepared without inoculation, i.e., raw linseed powder followed by roasted powder. Beverage prepared with raw seed powder had 1392 mg. kg⁻¹ of phytates. But, Oomah et al. (1996) analyzed eight linseed cultivars and reported that phytic acid content ranged between 22.8 to 32.5 g kg⁻¹ which seems high.
Table 1: Effect of linseed processing and bacterial inoculation on per cent phytate reduction.

| Treatments                  | Phytic acid (mg /100g) | % reduction in phytates |
|-----------------------------|------------------------|-------------------------|
| Raw powdered seed           | 1392a                  | -                       |
| Roasted powdered seed       | 1227b                  | 11.92i                  |
| Roasted powdered+ \textit{Lactobacillus acidophilus} | 0856c                  | 38.51a                  |
| Roasted powdered+ \textit{Bacillus mesentericus} | 0942c                  | 32.33b                  |
| Roasted powdered+ LAB -3    | 0950d                  | 31.76w                  |

Table 2: Effect of linseed processing and bacterial inoculation on bioavailability of iron and calcium.

| Treatments                  | Iron(mg /100 g) | Calcium(mg /100 g) |
|-----------------------------|-----------------|--------------------|
| Raw powdered seed           | 0.89a           | 125.03             |
| Roasted, powdered seed      | 1.20b           | 165.18             |
| Roasted powdered+ \textit{Lactobacillus acidophilus} | 4.40b       | 250.41             |
| Roasted powdered+ \textit{Bacillus mesentericus} | 2.07a       | 165.18             |
| Roasted powdered+ LAB -3    | 1.54c           | 208.67             |

The least quantity of phytates was recorded in the beverage inoculated with \textit{Lactobacillus acidophilus} followed by LAB-3 isolate. But, LAB-3 inoculated beverage was on par with \textit{Bacillus mesentericus} inoculated beverage. \textit{Lactobacillus acidophilus} inoculated treatment was significantly differing with other treatments by recording 38.50 % reduction in phytates. All the treatments differed significantly with each other. Didar (2011) investigated the effect of several lactic acid bacteria for the reduction of phytate. \textit{Lactobacillus plantarum} inoculation reduced phytate by 45%. Tang et al. (2010) reported 85 and 91% reduction in phytate contents using \textit{Lactobacillus acidophilus} and \textit{Lactobacillus plantarum} under \textit{in vitro} conditions. Osman (2011) prepared fermented beverage of pearl millet and studied the effect of fermentation on anti-nutritional factors. Results showed a significant reduction in phytic acid from 647.0 to 310.95 mg 100 g$^{-1}$ in fermented beverage after 24hrs of fermentation amounting to 52% reduction.

The highest bioavailable iron was recorded in case of \textit{Lactobacillus acidophilus} inoculated (4.40 mg /100 g) beverage sample followed by \textit{Bacillus mesentericus} (2.07 mg /100g) and LAB-3 (1.54 mg /100g). There was no significant difference between both \textit{B. mesentericus} and LAB-3 inoculated beverages with respect to bioavailable iron. Bioavailable iron was significantly higher in \textit{Lactobacillus acidophilus} inoculated sample than all other samples (Table 2). So from Table 1 and 2, a clear direct relationship can be established between bioavailability of iron and % reduction in phytates. These results uphold the fact that achieving reduction in phytates by fermentation can easily enhance the bioavailability of iron. A similar trend was observed with bioavailable calcium but, results were not significant.

Though linseed is a rich sink of iron and calcium, its consumption will not ensure in toto release of both minerals as it is rich in phytic acid also. Phytic acid being an antinutritional factor interferes with availability of minerals. So, an attempt to reduce phytic acid by microbial intervention in the form of fermentation will help in enhancing bioavailability of minerals.

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