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Effect of legume processing treatments individually or in combination on their phytic acid content

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The effect of some processing treatments, such as soaking, dehulling, boiling, autoclaving, microwave cooking, germination and fermentation with *Lactobacillus plantarum, L.bulgaricus, L.acidophilus and L.casei,* individually and in combination on the phytic acid content of soybean, mung bean and kidney bean was studied. Phytic acid content of raw soybean, mung bean and kidney bean was 35.01, 5.20 and 5.44 mg/g, respectively. Fermentation and germination individually and in combination with dehulling and cooking processes caused significant ($p<0.05$) decreases in phytic acid content more than that other of processing treatments. Among the three tested fermented seeds, kidney bean showed the greatest reduction in phytic acid 85.4 % compared to 77.0 % for soybean and 69.3 % for mung bean. However, *L.bulgaricus* was clearly the most effective one for decreasing phytic acid content during fermentation of different legumes.

Key words: Antinutritional factor, Legumes, Phytic acid, Cooking, Germination, Fermentation.

INTRODUCTION

Although legume seeds contain a moderately high amount of protein, calories, certain minerals and vitamins, their use in food and feed is still limited by the presence of several antinutritional factors (ANFs). These include tannins (Reddy et al., 1985), phytic acid (Urbano et al., 2000), trypsin inhibitors (Gupta 1987and Singh, 1988) and flatulence causing oligosaccharides (Singh, 1988; Udensi et al., 2007). Among all the antinutritional components, phytic acid is one of prime concern for human nutrition and health management (Kumar et al., 2010).

Phytic acid (myoinositol, 1, 2, 3, 4, 5, 6 hexakis-dihydrogen phosphate) and phytate (salts of phytic acid) are widespread in plant seed grains (also including cereals), roots, tubers (Graf, 1986; Lasztity and Lasztity, 1990) and legumes (Reddy et al., 1982). Phytate accumulates in the seeds during the ripening period and is the main storage form of both phosphate and inositol in plant seeds and grains (Loewus, 2002).

The phytate molecule is negatively charged at physiological pH and is reported to bind with essential, nutritionally important divalent cations such as Fe^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+}…. etc., and forms insoluble complexes, thereby making minerals unavailable for absorption (Rimbach et al., 1994). It also forms complexes with proteins and starch and inhibits their digestion (Oatway et al., 2001). The dephosphorylation of phytate is a prerequisite for improving nutritional value because removal of phosphate groups from the inositol ring decreases the mineral binding strength of phytate. These results increased bioavailability of essential dietary minerals (Sandberg et al., 1999).

Various food processing and preparation techniques, such as decortications, soaking, cooking, germination and fermentation, are the major efforts made to reduce the amounts of phytate in foods (Elmaki et al., 2007; Sangronis and Machado, 2007; Liang et al., 2008; Khattab and Arntfield, 2009; Wang et al., 2009; Kumar et al., 2010). The most effective treatments are fermentation (Marfo et al., 1990) and germination (Honke et al., 1998) but their application remains limited because of the additional workload they imply or the particular organoleptic properties they induce.

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ElMaki et al. (2007) studied the effects of soaking of white bean seeds on antinutritional factors and found that phytic acid contents of all cultivars were reduced. The reduction was between 4% and 16% after soaking whole seeds for 3 days at 30 °C.

Ologhobo and Fetuga (1984) also observed that autoclaving caused less loss of phytic acid in cooked beans compared to soaking and cooking by atmospheric boiling. Sharma and Kapoor (1996) found a marked reduction of phytic acid in pearl millet after autoclaving and reported that may be due to breakdown of phytic acid at high temperature. Similarly, cooking and autoclaving has been reported to cause decrease in phytic acid (Duhan et al., 1989 and Akinyele, 1989). Ordinary cooking of unsoaked and soaked white bean seeds brought about a significant decrease in phytic acid content when compared to the control (Elmaki et al., 2007).

Sangronis and Machado (2007) evaluate the effect of germination on some nutrients as well as on some antinutritional factors of white beans (*Phaseolus vulgaris L.*), black beans (*Phaseolus vulgaris L.*) and pigeon beans (*Cajanus cajan L. Mill sp.*) and found that the reduction of phytic acid was more than 40% for the three grains germinated and these variations in the content of nutrients and antinutrients of the germinated grains are attributed to the joint effect of the germination and previous soaking.

Shimelis and Rakshit (2007) also obtained a notable reduction (over 75%) in phytic acid in three kidney bean varieties after 4 days of germination.

The purpose of this work was to investigate the effect of individual or combined processing methods on the reduction/elimination of phytic acid content in soybean, mung bean and kidney bean. This would help in determine simple and cost-effective processing options for developing countries in order to improve the nutritional value of such beans.

Effects of selected strains of *L. acidophilus, L. buchneri, L. cellobiosus and L. fermentum* on oligosaccharide and phytate content of lupins were investigated by Camacho et al. (1991). Growth of *L .acidophilus, L .buchneri* was related to sucrose breakdown and decreases in phytate content**.**

Fermentation of brown beans (*phaseouls vulgaris*) that had been presoaked resulted in a reduction of phytate by 68 % after 48 h Gustafsson and Sandberg (1995).

MATERIALS AND METHODS

Materials

Seed samples

Mung bean (*Vigna radiata*), soybean (*Glycine max. L.*), and kidney bean (*Phaseolus vulgaris* L.), varieties were obtained from Agriculture Research Center (A.R.C), Ministry of Agriculture, Giza, Egypt. The seeds were thoroughly cleaned from dust and other extraneous materials prior to use.

Bacterial strains

Three strains of lactic acid bacteria (LAB), *Lactobacillus blugaricus* (EMCC1102), *L. casei* (EMCC1643) and *L. acidophilus* (EMCC1324) were obtained from the Egyptian Microbial Culture Collection (EMCC) at the Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. *Lactobacillus plantarum* (NRRL B-4004) was obtained from Northen Regional Research Laboratory, USA. These strains were selected according to their abilities to ferment legume seed extracts.

Chemicals

The chemicals used in this study (Trichloroacetic acid, ammonioum molybdate and sodium phytate) were purchased from Sigma Chemical Company, St. Louis, MO, USA.

Media

Lactobacilli broth

(MRS-broth) was used to activate as well as to prepare heavy suspension of the investigated bacterial strains. MRS agar was used to activate, maintain and enumerate the cultures of LAB strains according to the method of De Man et al. (1960).

Methods

Processing treatments

The processing treatments used for the reduction / elimination of phytate content were soaking (hydration), dehulling, germination, cooking (*i.e.*, boiling, autoclaving as well as microwave cooking) and fermentation with lactic acid bacteria. After each step for every particular processing treatment, samples were dried at 50 °C for 20 h. in a hot air oven and ground in an electric mill to pass from a 60 mesh sieve screen. The powdered samples were stored in plastic containers under refrigeration until analysis for their phytate content 4°C.

Soaking

The whole seeds of soybean, mung bean and kidney bean were soaked in distilled water (1:10 w/v) for 12, 18 and 24 h at room temperature (~25 °C).

Dehulling

Hulls were removed manually after soaking the seeds for different times according to El-Beltagy (1996).

Cooking methods

Seeds previously soaked in distilled water for 12 h were drained and rinsed three times with distilled water and then cooked by the methods described below El-Beltagy (1996).

Table 1. Effect of soaking on phytic acid content (mg/g dry weight basis) of soybean, mung bean and kidney bean.

Treatment	Soybean	I** %	Mung bean	R** %	Kidney bean	R** %
Raw	*35.01 [b] ± 0.28	-	5.20 [a] ± 0.05	-	5.44 [a] ± 0.01	-
Soaking						
12 h	37.04 [a] ± 0.14	5.8	4.56 [b] ± 0.15	12.3	4.59 [b] ± 0.02	15.6
18 h	37.30 [a] ± 1.32	6.5	4.47 [b] ± 0.02	14.0	4.37 [c] ± 0.07	19.7
24 h	37.52 [a] ± 0.87	7.2	4.28 [c] ± 0.02	17.7	4.14 [d] ± 0.03	23.9

Means in the same column with different letters are significantly ($p<0.05$) different
** I = Increase, R= Reduction

Boiling

Seeds were cooked in distilled water (100 °C) in the ratio of 1:10 (w/v) on a hot plate for 30, 60 and 90 min.

Autoclaving

Seeds were autoclaved using (SELECTA) at 15 atmospheric pressure (121 °C) in distilled water (1:10 w/v) for 10 min.

Microwave cooking

Seeds were placed in a Birex pot with distilled water (1:10 w/v), then cooked in a microwave oven (Sumsung 44L-900W) on high for 15 min (about 50% of the seeds were soft when felt between the fingers).

Germination

Different seeds were sterilized by soaking in ethanol for 1 min., soaked in distilled water (1:10 w/v) for 12 h at room temperature (≈ 25 °C), then kept between thick layers of cotton cloth and allowed to germinate in the dark for 1, 2,3,4 and 5 days. Germinated seeds were frozen for 12 h to stop the germination process.

Lactic acid fermentation

The milled seed samples were individually mixed with distilled water in a warring blender to obtain slurry ratio of 1:10 (dry legumes: water w/v). 50 ml of each prepared seed slurry were transferred to 100 ml flask and autoclaved for 15 min at 121 °C. The investigated LAB strains were activated on MRS agar for 48 h at 37 °C. The obtained growth was suspended in 5ml of MRS broth and re-incubated for 24 h at 37°C (activated microbial suspension). The flasks were inoculated with 0.5 ml of activated LAB strains (1 %) and incubated for 72 h at 37 °C.

Analytical methods

The phytic acid content in both raw and treated seed samples was determined according to the method of Mohamed et al. (1986) using chromogenic solution. The amount of phytic acid content was expressed as mg/ g dry sample.

Statistical analysis:

Results are expressed as mean value ± standard deviation (S.D) of three replicates. Data were statistically analyzed using analysis of variance and least significant difference using SAS (1985). Significant differences were determined at the 0.05 level of significance.

RESULTS AND DISCUSSION

The influence of soaking, dehulling, cooking, germination and fermentation as well as their combination on the level of phytic acid content presents in the seeds of soybean, mung bean and kidney bean were studied.

Effect of soaking

Generally, legumes are soaked in water overnight; phytate is water-soluble, so a considerable amount of phytate is removed into the water. In addition, this process also enhances the action of naturally occurring phytase in legumes (Kumar et al., 2010).

Phytic acid content in raw legume seeds ranged from 35.01 to 5.20 mg/g DM (Table1 and Figure 1). Concentration was the highest in soybean (35.01 mg/g), while the lowest was in mung bean (5.20 mg/g). Phytic acid content of soybean, mung bean and kidney bean were within the range of values reported by Chitra et al. (1995); Mubarak (2005); Elmaki et al. (2007) and Al-Numair et al. (2009).

Soaking of soybean for 12, 18 and 24 h increased the phytic acid content by 5.8, 6.5 and 7.2 %, respectively. Similar results were obtained by Egounlety and Arowh (2003). They found that soaking soybean for 12 - 14 h increased phytic acid content by 1.71%.

However, soaking of mung bean and kidney bean for different time periods minimized the level of phytic acid below the control value. On 12 h soaking, the percent loss was 12.3 and 15.6 % in mung bean and kidney bean, respectively. With an increase in the periods of

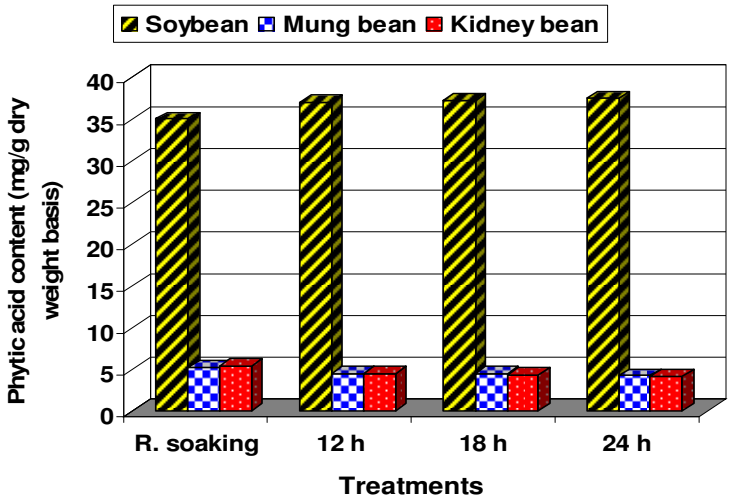

Figure 1. Effect of soaking on phytic acid content (mg/g dry weight basis) of soybean, mung bean and kidney bean.

soaking, *i. e.* 18 and 24 h, further reduction in phytic acid content was reached 17.7 and 23.9 % for mung bean and kidney bean, respectively, over the control values after soaking for 24 h.

The loss in phytates during soaking of the tested samples may be due to leaching of phytate ions into the soaking water under the influence of a concentration of gradient (difference in chemical potential), which governs the rate of diffusion, Similar results for reduction in phytic acid in soaked legumes have been reported earlier (Deshpande and Cheryan, 1983; Ologhobo and Fetuga, 1984).

Effect of different cooking methods

Cooking is usually done before the use of legumes in a human diet. This improves the protein quality by either destruction or inactivation of heat-labile antinutrtional factors (Mubarak, 2005).

The effect of different cooking methods (*i.e.* boiling, autoclaving and microwave) on the level of phytic acid content of some legumes was shown in Table 2.

Ordinary boiling of soaked tested seeds for different time periods brought about significant decrease in phytic acid content as compared with raw seeds. On 30 min boiling, the percent losses were 7.7, 15.4 and 16.5 % for soybean, mung bean and kidney bean, respectively. With an increase of boiling, *i.e.* 60 and 90 min, further reduction in phytate content of seeds was observed .Maximum phytate reduction (25.0 %) was occurred in mung bean after boiling for 90 min.

On other hand, there was a significant reduction in phytic content in different tested seeds after autoclaving for 10 min. It reduced phytate content by 16.3, 26.3 and 19.1 % for soybean, mung bean and kidney bean, respectively.

A significant ($p<0.05$) reduction in phytic acid content was noticed after microwave cooking for 15 min of different soaked seeds but this loss appeared to be significantly ($p<0.05$) less than that in seeds , which cooked by autoclaving for 10 min . Similar results were obtained by Mubarak (2005); Alajaji and El-Adawy (2006); Elmaki et al. (2007) and Shimelis & Rakshit (2007).

The apparent decrease in the content of phytic acid of legume seeds during cooking may be partly due to leaching into the cooking medium, degradation by heat or formation of insoluble complexes between phytate and other components, such as protein and minerals (Siddhuraju and Becker, 2001).

Phytic acid is relatively heat-stable, hence, significant and prolonged inputs of energy are requires for its destruction. Autoclaving for longer periods may results an additional phytate loss. Prolonged heat treatment reduced phytate content effectively (Sharma and Sehgal, 1992).

Effect of germination

Germination is a natural biological process of all superior plants by which the seed comes out of its latency stage. The process of germination has been developed in some countries as an alternative to defeat some of disadvantages associated with untreated grains, such as undesirable tables and smells, as well as the presence of trypsin inhibitors and phytates (Sangronis and Machado, 2007). Also, kumar et al. (2010) stated that during germination of cereals and legumes, phytate is degraded by intrinsic phytase. Plant seed utilize phytate as a

Table 2. Effect of different cooking methods on phytic acid content (mg/g dry weight basis) of soybean, mung bean and kidney bean.

Treatment	Soybean	R** %	Mung bean	R** %	Kidney bean	R** %
Raw Boiling	*35.01[a] ± 0.28	-	5.20[a] ± 0.05	-	5.44[a] ± 0.01	-
30 min	32.30[b] ± 0.61	7.7	4.40[b] ± 0.02	15.4	4.54[b] ± 0.01	16.5
60 min	30.93[c] ± 0.24	11.7	4.11[c] ± 0.06	21.0	4.51[b] ± 0.08	17.1
90 min	30.33[c] ± 0.76	13.4	3.90[d] ± 0.02	25.0	4.40[c] ± 0.06	19.1
Autoclaving 10 min	29.32[d] ± 0.34	16.3	3.83[e] ± 0.06	26.3	4.40[c] ± 0.05	19.1
Microwave 15 min	31.80[b] ± 0.26	9.3	4.20[c] ± 0.04	19.2	4.60[b] ± 0.01	15.4

* Means in the same column with different letters are significantly ($p<0.05$) different
** R=Reduction

Table 3. Effect of germination on phytic acid content (mg/gm) of soybean, mung bean and kidney bean.

Treatment	Soybean	R** %	Mung bean	R** %	Kidney bean	R** %
Raw Germination	*35.01[a] ± 0.28	-	5.20[a] ± 0.05	-	5.40[a] ± 0.01	-
1 day	31.20[b] ± 0.13	11.0	4.40[b] ± 0.03	15.4	4.40[b] ± 0.04	18.5
2 days	29.10[c] ± 0.18	17.0	4.20[c] ± 0.02	19.2	4.20[c] ± 0.12	22.2
3 days	28.20[d] ± 0.07	19.5	3.70[d] ± 0.01	28.8	4.10[c] ± 0.13	24.0
4 days	25.40[e] ± 0.21	27.4	3.45[e] ± 0.03	33.7	3.60[d] ± 0.03	33.3
5 days	23.70[f] ± 0.26	32.3	3.03[f] ± 0.01	41.7	3.50[d] ± 0.14	35.2

•Means in the same column with different letters are significantly
• ($p<0.05$) different
** R=Reduction

source of inorganic phosphate during germination and thus tend to increase palatability and nutritional value.

A significant reduction ($p<0.05$) was observed in phytic acid content of tested beans during germination for 5 days (Table 3). A loss of 11.0, 15.4 and 18.5 % was occurred during 24 h germination of soybean, mung bean and kidney bean, respectively, which was enhanced further with extending the period of germination. During the fifth day of germination period, phytic acid content of soybean, mung bean and kidney bean progressively

Table 4. Effect of fermentation with *L. plantarum* for 72 hours on phytic acid content (mg / gm) of soybean, mung bean and kidney bean.

Treatment	Soybean	R** %	Mung bean	R** %	Kidney bean	R** %
Raw Fermentation	*35.01 [a] ± 0.28	-	5.20 [a] ± 0.05	-	5.44[a] ± 0.01	-
24 h	30.01 [b] ± 0.23	14.3	2. 80 [b] ± 0.06	46.2	2.28 [b] ± 0.07	58.1
48 h	23.58 [c] ± 0.21	32.6	2. 60 [c] ± 0.03	50.0	2.05 [c] ± 0.05	62.3
72 h	15.90 [d] ± 0.14	54.6	2. 30 [d] ± 0.06	55.8	1.60 [d] ± 0.06	70.6

Means in the same column with different letters are significantly ($p<0.05$) different
** R=Reduction

decreased from 35.1 to 23.70, 5.2 to 3.03 and 5.4 to 3.5 mg/ g, respectively.
Reduction of phytate content due to germination was higher in mung bean (41.7 %) than in soybean and kidney bean (32.3 and 35.2 %, respectively).
Previous reports on the effect of germination on phytic acid in beans indicated that as a result of increased enzymes activity, 20-70 % or more of phytic acid is hydrolyzed during germination (Reddy et al., 1982.; Desphande, 1985).Similarly, Shimelis and Rakshit (2007) also reported that a notable reduction (over 75%) in phytic acid of three kidney bean varieties after 4 days of germination was obtained.

Effect of fermentation

Food fermentation is a microbial and enzymatic method for food processing to achieve prolonged shelf-life. Lactic fermentation leads to lowering pH as a consequence of bacterial production of lactic and acetic acids, which is favourable for phytase activity, resulting in lowering of phytates (Lopez et al., 1983).
Therefore, the effect of fermentation of legumes (1seed:10water) with *L. plantarum , L. bulgaricus, L. acidophilus and L. casei* at 37 ° C for 72 on their phytic acid content was studied and the results are given in Tables (4, 5, 6 and 7 and Figure. 2, 3, 4 and 5) .
Compared to raw legume seeds, fermentation with different LAB strains decreased phytic acid content. As the period of fermentation increased, a significant decrease ($p<0.05$) in phytic acid content occurred with the different tested strains. A significant ($p\leq 0.05$) decrease in phytic was firstly observed at 24 h fermentation with *L. plantarum* (14.3, 46.2 and 58.1 % for soybean, mung bean and kidney bean, respectively) and further significant reductions were observed at 72 h (54.6, 55.8 and 70.6 %) for the same sample (Table 4 and Figure 2). The same pattern was found for the other tested strains (Tables 5, 6 and 7& Figure , 3, 4 and 5).
Among the four tested fermented seeds, kidney bean showed the greatest reduction in phytic acid (68.9 to 85.4 %) compared with 39.2 to 77.0 %for soybean and 55.8 to 69.2 % for mung bean with the different tested lactic bacteria after 72 h of fermentation.
Among the four tested LAB strains *L. bulgaricus* was clearly the most effective one for decreasing phytic acid content during fermentation of different investigated legumes. After 72 h of fermentation with L. *bulgaricus*, phytate content was reduced by 77, 69.2 and 85.4 % for soybean, mung bean and kidney bean, respectively.
Generally, fermentation is known to cause a greater reduction in phytic acid to the low pH of fermented product, which is considered as optimum for phytase activity (El-Hag et al., 2002).
A decreasing effect of lactic acid fermentation on the phytate content in sorghum has been demonstrated earlier (Svanberg et al., 1993). Both cereal and microbial phytase can contribute to reduce in phytate during fermentation process (Dhankher and Chauhan, 1987**.;** Reddy and Pierson, 1994).

Effect of combined processing treatments

Most researchers have studied the effect of individual processing methods on antinutrtional factors , but information on the effect of combined methods in comparison with the most effective individual one for reducing phytic acid content of legumes are studied (Table 8).
Soaking as well as dehulling of seeds contributed significantly ($p<0.05$) towards lowering down phytic acid content of the tested seeds. No doubt dehulling of soaked

Table 5. Effect of fermentation with *L. bulgaricus* for 72 hours on phytic acid content (mg / gm) of soybean, mung bean and kidney bean.

Treatment	Soybean	R** %	Mung Bean	R** %	Kidney bean	R** %
Raw Fermentation	*35.01 [a] ± 0.28	-	5.20 [a] ± 0.05	-	5.44 [a] ± 0.01	-
24 h	18.00 [b] ±0.76	48.6	2. 90 [b] ± 0.07	44.2	2.40 [b] ± 0.1	55.9
48 h	14.80 [c] ±0.25	57.7	2. 30 [c] ± 0.07	55.8	1.60 [c] ± 0.1	70.6
72 h	8.04 [d] ±0.21	77.0	1.60 [d] ± 0.12	69.2	0.79 [d] ± 0.1	85.4

*Means in the same column with different letters are significantly ($p<0.05$) Different
** R=Reduction

Table 6. Effect of fermentation with *L. acidophilus* for 72 hours on phytic acid content (mg / gm) of soybean, mung bean and kidney bean.

Treatment	Soybean	R** %	Mung bean	R** %	Kidney bean	R** %
Raw Fermentation	*35.01 [a] ± 0.28	-	5.20 [a] ±0.05	-	5.40 [a] ± 0.01	-
24 h	23.10 [b] ± 0.92	34.0	2. 60 [b] ±0.02	50.0	2.60 [b] ± 0.03	51.9
48 h	18.10 [c] ± 0.59	48.3	2. 40 [c] ±0.09	53.8	2.10 [c] ± 0.07	61.1
72 h	11.90 [d] ± 0.75	66.0	2.00 [d] ±0.06	61.5	1.68 [d] ± 0.09	68.9

Means in the same column with different letters are significantly ($p<0.05$) different
** R=Reduction

Table 7. Effect of fermentation with *L. casei* for 72 hours on phytic acid content (mg / gm) of soybean, mung bean and kidney bean.

Treatment	Soybean	R** %	Mung Bean	R** %	Kidney bean	R** %
Raw Fermentation	*35.01 [a] ± 0.28	-	5.20 [a] ± 0.05	-	5.40 [a] ± 0.01	-
24 h	26.70 [b] ± 0.42	23.7	2. 60 [b] ± 0.04	50.0	2.50 [b] ± 0.07	53.7
48 h	25.95 [c] ± 0.2	25.9	2. 60 [b] ± 0.26	50.0	1.90 [c] ± 0.02	64.8
72 h	21.30 [d] ± 0.49	39.2	1.70 [c] ± 0.1	67.3	1.20 [d] ± 0.04	77.8

* Means in the same column with different letters are significantly ($p<0.05$) different
** R=Reduction

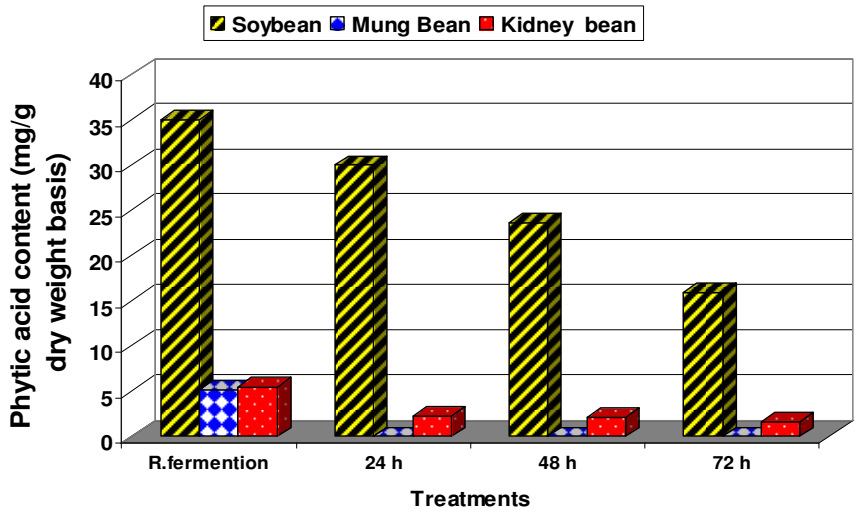

Figure 2. Effect of fermentation with *L. plantarum* for 72 hours on phytic acid content (mg / gm) of soybean, mung bean and kidney bean

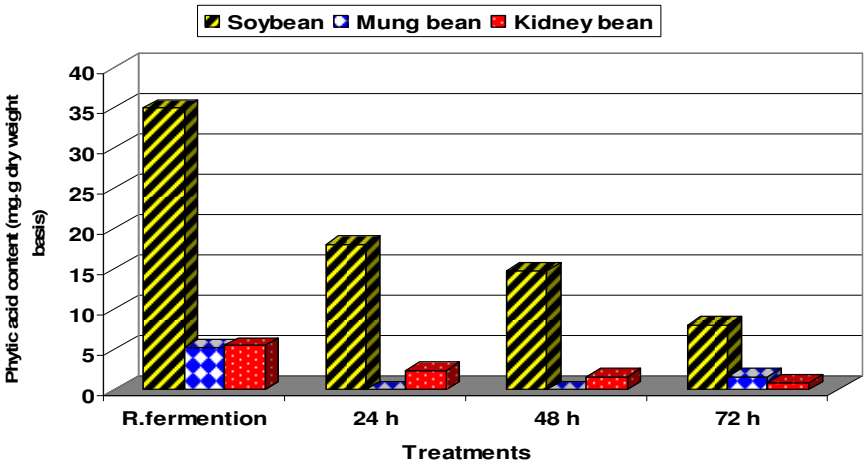

Figure 3. Effect of fermentation with *L. bulgaricus* for 72 hours on phytic acid content (mg / g) of soybean, mung bean and kidney bean.

soybean lowered the phytic acid content but the loss appeared to be less than in other tested seeds because soaking of soybean significantly increased phytic acid content as mentioned before (Table 1 and Figure.1).

A 7.5 and 6.3 % of phytate was lost as the result of dehulling of soaked mung bean and kidney bean, showing the importance of dehulling treatment in bean processing .Similar results were obtained by Bishnoi et al. (1994).

Germination alone was more effective for lowering heat-stable phytic acid content than cooking methods .However the impact of germination followed by dehulling and cooking caused significant ($p< 0.05$) decreases in phytic acid content by 45.7 – 53.5 % for different legumes compared to 32.3 and 41.7 % for germinated ones.

However, fermentation with *L. bulgaricus* of autoclaved samples was sharply decreased phytic acid content in different tested legumes. Results also indicate that reduction of phytic acid content using different processing treatments is going to depend on the type of legume. Among the three tested seeds, autoclaved kidney bean showed the highest reduction in phytic acid (85.4%) compared to 77.0 and 69.2 % for autoclaved soybean and mung bean, respectively, when a combined with fermentation by *L. bulgaricus* for 72 h.

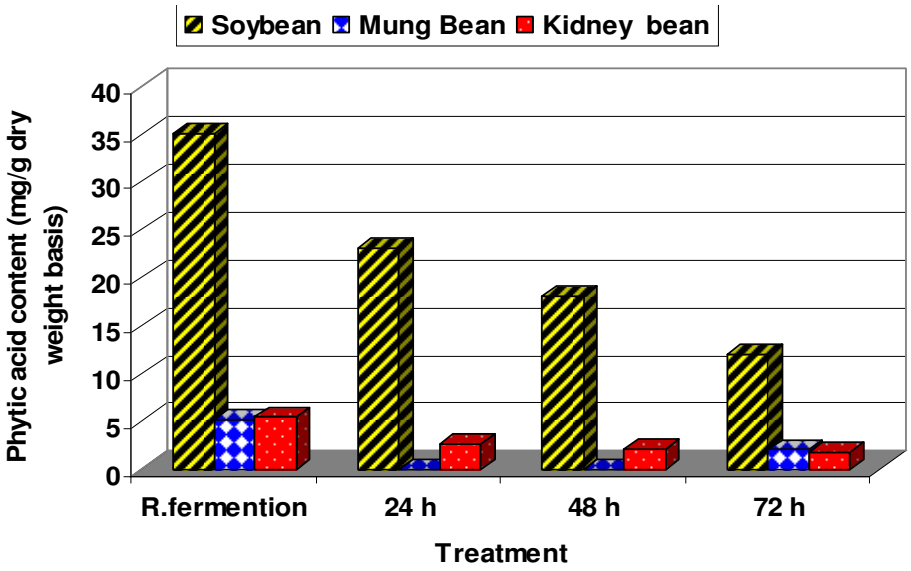

Figure 4. Effect of fermentation with *L. acidophilus* for 72 hours on phytic acid content (mg / gm) of soybean, mung bean and kidney bean.

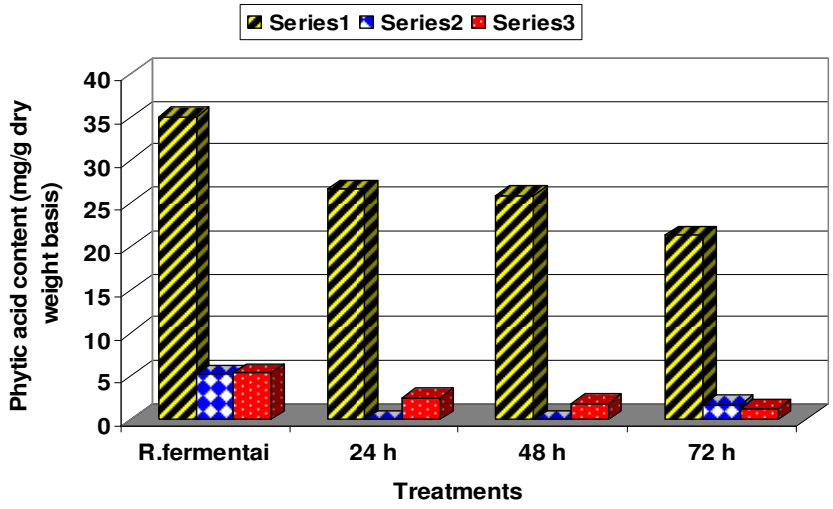

Figure 5. Effect of fermentation with *L. casei* for 72 hours on phytic acid content (mg / gm) of soybean, mung bean and kidney

Similar results were obtained by Sharma and Kapoor (1996); they reported that germination and autoclaving were the most effective processing treatments to reduce phytic acid content in pearl millet. They also reported that phytic acid was completely eliminated after fermentation in some of samples especially in soaked, debarnned and germinated ones. Phytic acid content acceptable after the effective treatment.

CONCLUSION

Fermentation and germination individually and in combination with dehulling and cooking processes caused decreases in phytic acid content more than that other of processing treatments. Fermented seeds, kidney bean showed the greatest reduction in phytic acid. However, *L. bulgaricus* was clearly the most effective one

Table 8: Effect of different processing treatments on phytic acid content (m g/g) of soybean mung bean and kidney bean.

Treatments	Soybean	R** %	Mung bean	R** %	Kidney bean	R** %
Raw	*35.01^{Ab} ± 0.28	-	5.20^{Ba} ± 0.05	-	5.40^{Ba} ± 0.01	-
Soaking (24 h)	37.52^{Aa} ± 0.87	7.2	4.28^{Bb} ± 0.02	17.7	4.14^{Bc} ± 0.03	23.3
Soaking (24 h) + Dehulling Cooking	34.70^{Ab} ± 0.42	1.0	3.89^{Bd} ± 0.03	25.2	3.80^{Bd} ± 0.03	29.6
Boiling (90 min)	30.33^{Ac} ± 0.76	13.4	3.9^{Bc} ± 0.02	25.0	4.40^{Bb} ± 0.06	18.5
Autoclaving (10 min)	29.32^{Ad} ± 0.34	16.3	3.83^{Cd} ± 0.06	26.3	4.40^{Bb} ± 0.05	18.5
Germination	23.70^{Ae} ± 0.26	32.3	3.03^{Ce} ± 0.01	41.7	3.50^{Be} ± 0.14	35.2
Germination + dehulling	20.50^{Ag} ± 0.15	41.4	2.70^{Cg} ± 0.03	48.1	3.10^{Bf} ± 0.25	42.6
Germination + boiling(30 min)	22.89^{Af} ± 0. 1	34.6	2.81^{Cf} ± 0.02	46.0	3.43^{Be} ± 0.02	36.5
Germination+ dehulling + boiling(30 min)	19.00^{Ah} ± 0. 2	45.7	2.42^{Ch} ± 0.004	53.5	2.84^{Bg} ± 0.02	47.4
Autoclaving + Fermentation by *Lactobacillus bulgaricus*	8.04^{Ai} ±0.21	77.0	1.60^{Bi} ± 0.12	69.2	0.79^{Ch} ± 0.1	85.4

* Means in the same column with different small letters are significantly ($p<0.05$) different
* Means in the same raw with capital different letters are significantly ($p<0.05$) different
** R = Reduction

for decreasing phytic acid content during fermentation of different legumes

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