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EFFECT OF SELECTED FOOD ADDITIVES ON PHYTIC ACID CONTENT OF SOYBEAN DURING SOAKING

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The effect of food additives of sodium chloride (NaCl) and sodium bicarbonate ($NaHCO_3$) was studied on the hydrolysis of phytic acid in soybean, soaked in water and in solutions of pH4 and 6 at 30°C for 6 and 12 h. Four varieties of soybean V1 (B1-080/36), V2 (AGS-62), V3 (G1-0031) and V4 (EPPS) were selected. The amount of phytic acid in V1, V2, V3, and V4 of soybean was 12 mg, 11 mg, 13 mg and 12.5 mg/g respectively. Soaking of soybean flour for 6 and 12 h in water and in pH4 and 6 solutions have significantly reduced ($P < 0.05$) the levels of phytic acid. The effect of pH6 solution was most effective, while the effect of water and pH4 was similar in lowering the phytic acid in soybean. The presence of mixed food additives (1% NaCl + 2% $NaHCO_3$) in water, and pH4 and 6 solutions, reduced the level of phytic acid in soybean to 38%, 52% and 56% for 6 h, and 48%, 55% and 68% for 12 h in respective solutions. It was observed that soaking of soybean flour in pH 6 solution in the presence of NaCl and $NaHCO_3$ phytase enzyme of the flour is well activated to hydrolyse phytic acid. These results suggest that soaking with sodium chloride and sodium bicarbonate can reduce the phytic acid in soybean flour. This treatment can improve the nutritional value of the soyabean flour, which is used in various food products of dairy and confectionery etc.

Key words: Food additives, Phytic acid, Soyabean, Soaking.

Introduction

Legumes are the important source of dietary protein for the large segment of the world population, especially in areas where use of animal protein in human diet is limited due to its non-availability, or cultural and religious constraints. In addition to protein, legumes also provide energy, minerals, and some vitamins. The quality of protein depends on their amino acids composition and legumes are generally rich in lysine, threonine, valine, leucine and isoleucine. This pattern of amino acids in legumes increases their importance as a supplement for cereal diets, which are usually deficient in lysine (Mtenga and Sugiyamma 1974). In plants, number of chemical compounds are synthesized which can adversely affect the quality of plant products used as human and animal food. One of the most important of such anti-nutritional factors is the phytic acid, which occurs in cereals, legumes and oilseeds. The presence of phytic acid decreases the bioavailability of many essential minerals and protein (Maga 1982).

Soybean is a leguminous crop extensively grown for the production of edible oils and proteins. The soybean belongs to the family Leguminoseae, subfamily Papilionoideae, and the genus *Glycine.* Soybean seeds vary in color from yellow, green or brown to black, and are either solid colored or bi-colored. They are spherical, elongated, or oval in shape. Soybean originated in China, where records of it go back to 2838 B.C. and it was spread to other parts of the world very late. The present commercial soybean cultivars were introduced in Pakistan in early of the year 1960 from the USA for experimental purposes. Cultivation of soybean on a commercial scale in the province of NWFP was reported in the years of 1970 - 1971, in Sind province in 1975 - 1976, and in Punjab province in 1983. In the province of Balochistan, it is still in the experimental stage. The total production of soybean in Pakistan is 7228 tones and in NWFP 6410 tones (Agriculture Statistics of Pakistan, 1994 - 1995).

To improve the nutritional quality of soybean by elimination the anti-nutritional factors, the influence of food additives of sodium biocarbonate and sodium chloride in soaking was studied on phytic acid in soybean flour soaked in water and various pH solutions at 30°C for different times.

*Chemistry of phytic acid.*The utilization of protein in human body depends on the quality of protein that is the presence of essential amino acids, digestibility and anti-nutritional factors including phytate. Phytic acid is the normal constituent of cereals and leguminous seeds and usually occurs in the form of phytate. This represents a complex class of naturally occurring compounds that can significantly influ-

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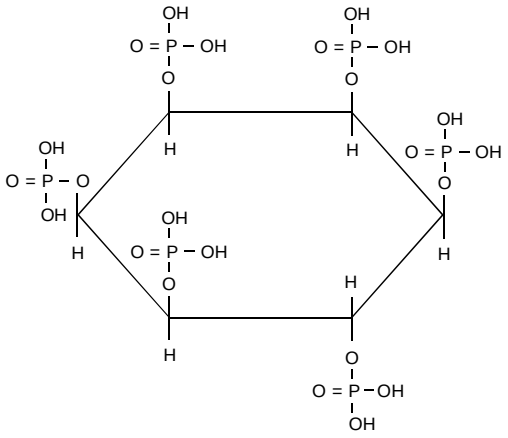

Fig. 1 Structure of phytic acid ($C_6H_{24}0_{27}P_6$) suggested by Neuberg (1908).

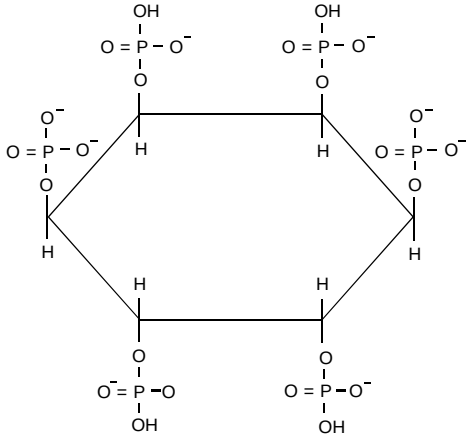

Fig. 2 Structure of phytic acid ($C_6H_{18}O_{24}P_6$) proposed by Anderson (1914).

ence the functional and nutritional properties of foods. Phytic acid is commonly called myo-inositol hexaphosphoric acid or 1, 2, 3, 4, 5, 6-hexakis (dihydrogen phosphate) myo-inositol. Phytic acid can interact with various minerals make insoluble complexes thus decreasing the bio-availability of many essential minerals such as calcium, phosphorous, magnesium, iron and zinc and also protein in human diet (Maga 1982). The interaction of phytic acid with protein is depend on the pH of the medium. At pH level below the isoelectric point of protein, phytate binds directly to protein action and at pH above the IP it binds to the protein through an alkaline earth metal (Cheryan 1980).

In legumes, a level of phytic acid is approximately up to 5% by weight. In soybean, 70 - 80% of the phosphorous is present in the form of phytic acid, the phosphoric acid ester of inositol. The interaction between phytic acid, mineral and/or protein appears to be primary factor responsible for its adverse nutritional effects in high phytate diets. The mechanism for this interaction can be explained by the structure of phytic acid. Neuberg (1908) proposed phytic acid chemical formula $C_6H_{24}O_{27}P_6$ having three P-O-P linkages between pairs of adjacent phosphates (Fig 1). Anderson (1914) proposed phytic acid chemical formula $C_6H_{18}P_6O_{24}$. (Fig 2). Gosselin and Coughlan (1953) studied calcium-phytic acid interaction using an ion-exchange equilibrium technique and concluded that there is P-O-P within the phytic acid molecules, in accordance with Neuberg structure. Fischer and Kurten (1932) also came to similar conclusion.

Materials and Methods

(1) Sample collection. For this research project, the following approved varieties of soybean of the year 1996, were collected from the Malakandher Farm, of NWFP Agricultural University, Peshawar:

(a) V1 (B1 - 080/36) (b) V2 (AGS - 62)
(c) V3 (GL - 0031) (d) V4 (EPPS)

The study was carried out in the Laboratory of the department of Food Science and Technology, NWFP Agricultural University, Peshawar.

(2) Sample preparation. The sample was cleaned off from all the impurities and sorted out for uniform shape and size. The seeds were ground by using a mesh of 40mm sieve. The ground and sieved samples were packed in plastic bags and stored at room temperature for subsequent chemical analysis.

(3) Protein analysis. Duplicate sample (1.5g) was placed in a digestion flask, 5g of digestion mixture and 30 ml of concentrated H_2SO_4 were added. The digestion flasks were then transferred to digestion assembly and the temperature adjusted to 100˚C which was increased gradually to 400˚C. The digestion was continued until the solution in the digestion flask was cleared and all organic matter oxidized. The digestion was completed within 2 h, after which the flasks were cooled for one h.

Distillation of the digest was performed by Micro-Kjeldahl apparatus using 5 ml from digestion flask and 5 ml NaOH and distilled water added. The sample was distilled and distillate was collected in a conical flask containing 5 ml of 2% boric acid solution with mixed indicator of methyl red. The distillation was completed in 5 minutes. The pinkish color was changed during distillation. Distillate collected in conical flask was titrated against standard HCl (0.01 N) solution. End point was noted when pink color appeared. Milliliters (ml) of standard HCl solution used were noted and percent crude protein was calculated as follows:

$$\% \text{ protein} = \frac{(S\text{-}B) \times 0.014 \times D \times 100 \times 6.26}{\text{Weight of sample x V}}$$

S = Volume of standard acid used for sample titration.
B = Volume of standard acid used for blank titration.
N = Normality of the acid used.
D = Sample dilution after digestion.
V = Volume of the digest taken for distillation after dilution.
0.014 is the equivalent weight of nitrogen and 6.25 is the general nitrogen to protein conversion factor.

(4) Food additives, and soaking of the soybean sample. The ground sample of soybean (approx.10 g) was soaked in 100 ml beaker in water and solutions of pH 4 and 6, with and without food additives of sodium chloride (1%) and sodium bicarbonate (2%) separately and mixed of both at 30°C for 6 and 12 hours.

(5) Phytic acid determination. Method developed by Huag and Lantzch (1983) was used for the determination of phytic acid. This method is based on phytic acid precipitation with an acid iron-III solution of known iron contents.

Preparation of solution: (i) HCl (2N). HCl (167.4 ml) was dissolved in distilled water to make a final volume of 1 litre.*(ii) Ferric solution.* Ammonium iron-III-sulphate [$NH_4(SO_4)_2$.12 H_2O] (2 g) was dissolved in 100 ml of 2N HCl and the volume was made to 1 liter with distill water in volumetric flask. *(iii) 2,2 Bipyridine solution.* 2,2, bipyridine (5 g) dissolved in 5 ml of thioglycollic acid ($HSCH_2COOH$) and then the volume were made up to 500ml in volumetric flask. *(iv) Phytic acid solution.* Phytic acid (Purity 98%) 0.15 g was dissolved in water and the volume was made to 100 ml in volumetric flask.

(6) Statistical analysis. The data were analyzed statistically by the procedure of analysis of variance by using Randomized Complete Block Design (RCBD) with split plot design. The mean were separated by applying the Least Significant Differences (LSD) test. This test is recommended by Snedecor and Cochran (1967).

Results and Discussion

(1) Analysis of soybean for protein and phytic acid. Four varieties of soybean were analyzed for the protein and phytic acid contents. The name of these varieties are V1 (B1 -080/36), V2 (AGS - 62), V3 GL - 0031 and V4 EPPS. Table 1 shows the protein and phytic acid contents analyzed in these varieties of soybean. The data revealed that the protein content in V1, V2, V3 and V4 varieties is 39.8%, 37.3%, 41.0% and 40.4%, respectively. To analyzed phytic acid the standard calibration curve was prepared from standard phytic acid according to the method of Haug and Lantzch (1983) (Fig 1). The amount of phytic acid content in V1, V2, V3 and V4 varieties of soybean is 12 mg, 11 mg, 13 mg and 12.50 mg/g, respectively (Table 1).

Table 1
Protein and phytic acid contents of whole soybean flour

Variety	% Protein	Phytic acid (mg/g)
(V1) B1-080/36	39.8%	12.00
(V2) AGS-62	37.3%	11.00
(V3) GL-0031	41.0%	13.00
(V4) EPPS	40.4%	12.50

It was reported that soybean is composed of three major components, the hull, cotyledon and hypocotyl, in which protein is approximately 8%, 90% and 2% respectively. Typical composition of soybean was found to be, protein 42% fat 20%, total carbohydrate 35%, ash 5.0% and crude fiber 5.5%. One third of soybean is carbohydrates, which included various polysaccharides and sucrose. The balance of the materials present in soybeans was described as ash which included many minerals (FAO 1977). Rham and Jost (1979) reported the nutrient composition of soybean as moisture 7.6%, protein 50.0%, fat 0.9%, phytate 1.5%, Ca 0.24%, Mg 0.32%, Na 0.01%, K 0.05%, P 0.7% and ash 4.2%. Andrew and Winton (1965) described that soybean seed contained 5.85 - 19.27% moisture, 26.25 - 40.22% protein, 12.27 - 19.0% fat, 3.07 - 5.40% ash, 2.45 - 6.13% crude fiber and 26.17 - 32.84% nitrogen free extract. Rehman and Nawaz (1975) determined the protein and oil contents of various varieties of soybean and concluded that the protein content was lowest 37.75% in Bragg and highest 43.73% in Lee variety. The mean protein content of the varieties was 40.35% over 500 lines of soybean seed and reported that the protein and oil contents ranged from 30 to 46% and 12 to 24% respectively. Cartter and Hopper (1942) had earlier reported the average composition of 10 common varieties of soybean, which contain 42.78% protein, 19.83% fat, 4.99% ash, 5.52% crude fiber and 7.97% sugar. Ranjana *et al* (1988) and Clark and Proctor (1994) reported 54.93, 46.84 and 50.20% protein and 6.15, 7.65 and 5.35% ash in soybean flour.

Lolas *et al* (1976) reported that the phytic acid content of 15 soybean varieties ranged from 1.00 to 1.47% dry weight which represented between 51.4 and 57.1% of the total phosphorous. They also evaluated phytic acid levels in 19 oat varieties in a range of 0.84-1.01% based on dry weight and proposed that phosphorous measurement could estimate the amount of phytic acid content. He also found a phytic acid 0.62-1.35% dry weight in whole kernels of wheat, whereas, the bran portion had phytic acid levels ranging from 4.59 to 5.52%, demonstrating that foods containing added wheat bran could have unexpectedly high levels. Chen and Pan (1977) reported that soybean and two varieties of pea seed (Dwarf and Early Alaska)

contained 2.48, 1.13 and 1.86 mg/g phytate respectively. After germination for 5 days and extracted three times each with 5 ml of 0.5 N HCl for 40 minutes, the phytate decreased to 1.94, 0.59 and 1.20 mg/g respectively.

Ganesh Kumar *et al* (1978) reported that green gram, cowpea, and chickpea contain phytic acid 0.65, 0.43 and 0.28%, respectively. The extraction pattern of phytin-P in aqueous for uncooked legumes were 0.124, 0.090 and 0.056%, respectively. The maximum extraction of phytin-P by hydrochloric acid (0.5 N) media for uncooked legumes was 0.185, 0.123 and 0.078% respectively and for the cooked legumes was 0.150, 0.090 and 0.078%, respectively. Cooking resulted in the decrease of both water and acid extractable phytin-P, but the loss of acid extractable was much less then water extractable ones.Gad *et al* (1982) found that phytic acid content in Broadbean seed is 274.9 mg/100g, peas 222.7 mg/100g, fenugreek 190.2 mg/100g, chickpea 184.5 mg/100g, lentil 149.7 mg/100g, and lupine 91.9 mg/100g respectively. They further reported that beans contained total phosphorus 518.2 mg/100g, peas 345.2 mg/100g, lentil 357.5 mg/100g and lupine 340.1 mg/100g respectively.

(2) The influence of food additives, pH, temperature and times on phytic acid during soaking of soybean. The effect of various factors such as (i) Food Additives (ii) pH (iii) Temperature and (iv) Times were studied on the hydrolysis of phytic acid content in soaking of four varieties of soybean. The research was carried out in the model system, in which, the ground sample of soybean was soaked in tap water and in solution of pH 4 and pH 6 at 30˚C for 6 and 12 h; (a) with no food additives, (b) with 1% sodium chloride, (c) with 2% sodium bicarbonate and (d) with 1% sodium chloride + 2% sodium bicarbonate mixture.

The results in Table 2 shows the effect of soaking on phytic acid content in soybean in tap water, pH4, pH6 solutions (with no food additives) at 30˚C for 6 and 12 h, and the analysis of variance is given in Appendix-I. Soaking for 6 h at 30˚C, in V1 of soybean the phytic acid was reduced from its original level (12 mg/g) in water, pH 4 and pH 6 solutions to 11.54 mg, 11.46 mg and 10.50 mg/g and in V2 (11 mg/g) to 10.64 mg, 10.41 mg and 9.73 mg/g, respectively. Similarly in V3, the phytic acid (13 mg/g) was reduced to 12.65 mg, 12 mg and 11.36 mg/g and in V4 (12.50 mg/g) to 11.85 mg, 11.58 mg and 10.86 mg/g, respectively. Soaking the soybean for 12 hours at 30˚C, in tap water, pH 4 and pH 6 solutions, the phytic acid in V1 was reduced to 11.28 mg, 10.88 mg and 9.88 mg/g, and in V2 to 10.54 mg, 9.96 mg and 9.48 mg/g respectively. In V3 the phytic acid reduced to 11.93 mg, 11.64 mg and 10.56 mg/g and in V4 11.46 mg, 11.30 mg and 10.36 mg/g respectively. Among these varieties, the highest reduction in the level of phytic acid was found at pH 6, 12 h soaking. Statistically, soaking the soybean all four varieties in tap water, pH 4 and pH 6 solutions for both times 6 and 12 h, phytic acid was significantly reduced ($P<0.05$).

Table 2

Retention of phytic acid in soybean flour (mg/g) after soaking at 30˚C for different times with no food additives. Before soaking phytic acid level in soybean was in V1 (12 mg/g), V2 (11 mg/g), V3 (13 mg/g) and V4 (12.5 mg/g)

Variety	Time(h)	Phytic acid (mg/g) Soybean flour			Mean
		Water	pH4	pH6	
V1	6	11.54F	11.46G	10.50Q	11.17C
	12	11.28J	10.88K	9.88U	10.68F
V2	6	10.64N	10.41R	9.73V	10.26G
	12	10.54P	9.96T	9.08W	9.86H
V3	6	12.65A	12.00B	11.36H	12.00A
	12	11.93C	11.64D	10.56O	11.38B
V4	6	11.85M	11.58E	10.86L	11.10D
	12	11.46G	11.30I	10.36S	11.04E
Mean		11.486A	11.154B	10.291C	

Figures showing the same letter(s) are statistically not different from one another (LSD Test).

Table 3

Retention of phytic acid in soybean flour (mg/g) after soaking at 30˚C for different times with 1% sodium chloride (NaCl). Before soaking phytic acid level in soybean was in V1 (12mg/g), V2 (11 mg/g), V3 (13 mg/g) and V4 (12.5 mg/g)

Variety	Time(h)	Phytic acid (mg/g) Soybean flour			Mean
		Water	pH4	pH6	
V1	6	10.04H	10.68K	10.08P	10.60D
	12	10.84J	10.30N	9.43T	10.52F
V2	6	10.24O	9.72S	9.34U	9.76G
	12	9.92Q	9.40T	8.64V	9.32H
V3	6	12.00A	11.51C	10.86I	11.46A
	12	11.66B	11.23F	10.30N	11.06B
V4	6	11.45D	11.10G	10.62L	11.06C
	12	11.26E	10.68K	9.80R	10.58E
Mean		11.051A	10.578B	9.883C	

Figures showing the same letter(s) are statistically not different from one another (LSD Test).

The results in Table 3 show the effect of soaking on phytic acid content in soybean in tap water, pH 4 and pH 6 solutions in the presence of 1% sodium chloride at 30˚C for 6 and 12 h. The analysis of variance is given in Appendix-II. Soaking for 6 h, in V1 of soybean, the phytic acid was reduced from its original level (12 mg/g) in water, pH 4 and pH 6 solutions to

11.04 mg, 10.68 mg and 10.08 mg/g and in V2 (11 mg/g) to 10.24 mg, 9.72 mg and 9.34 mg/g, respectively. Similarly in V3, the phytic acid (13 mg/g) was reduced to 12 mg, 11.51 mg and 10.86 mg/g and in V4 (12.50 mg/g) to 11.45 mg, 11.10 mg and 10.62 mg/g, respectively. Soaking the soybean for 12 hours at 30˚C, in tap water, pH 4 and pH 6 solutions the phytic acid in V1 was reduced to 10.84 mg, 10.30 mg and 9.43 mg/g and in V2 to 9.92 mg, 9.40 mg and 8.64 mg/g respectively. In V3, the phytic acid reduced to 11.66 mg, 11.23 mg and 10.30 mg/g and in V4 11.26 mg, 10.68 mg and 9.80 mg/g respectively. Statistically, soaking all the four varieties of soybean in tap water, pH 4 and pH6 solutions in the presence of 1% sodium chloride for both times 6 and 12 h at 30˚C, phytic acid significantly reduced ($P < 0.05$). The effect of other processing on phytic acid content in various food has been reviewed by other workers.

Lyer *et al* (1980) found that when Pinto, Great Northern and Red kidneybeans were soaked in distilled water for 18 h at room temperature the phytate content of beans was appreciably reduced to 52.7, 69.6 and 51.7% respectively. However, they noticed a somewhat lessen phytate hydrolysis when the beans were soaked in a mixed solution (2.5% sodium chloride + 1.5% sodium bicarbonate + 9.5% sodium carbonate + 1.0% sodium tripolyphosphate) at pH7 and room temperature of 21˚C.

Prattley *et al* (1982) reported that in commercial concentrate of soya isolates free phytic acid concentration was obtained at pH 5. On neutralization of the isolates, the formation of phytate-protein complexes increased. Under alkaline conditions, divalent cations (e.g. Ca, Mg, and Zn) interact with phytic acid and mediate in protein-phytate interaction. Under acid conditions the protein form an insoluble complex with phytic acid. Calcium produce different effect at higher pH (>6). Soluble protein-calcium phytic acid complexes were formed which were less stable to heat and dissociation above pH 10. Since this interaction occurred only in the presence of calcium, a salt linkage is implicated in which divalent cations bind to the phytic acid in the form of a complex. Prattley *et al* (1982) therefore, proposed that either addition of divalent cations at low pH could effectively remove phytate from soya products by ultra filtration.

Chompreeda and Fields (1984) have reported that autoclaving the soybean meal at 121˚C for 30 min reduced phytate content by 17.5%. The corn meal which containing 220.3 mg/100g phytate phosphorous, after fermentation at 32˚C for 4 h was decreased by 77.7%, whereas the same fermentation in meal mixture (90% corn-10% soybean), (85% corn-15% soybean) and (80% corn-20% soybean), decreased the phytate phosphorus by 44.4%, 50.2% and 35.5% respectively.

Serriano *et al* (1985) reported that phytic acid in rape seed flour was reduced at pH 5.15 with subsequent dialysis or by phytase treatment. The effect of phytate reduction on the rate and extent of protein and amino acid digestibilities were determined using *in vitro* pepsin-pancreatin proteolysis method. Phytic acid reduction (51%) increased the release of many amino acids. Further reductions of phytic acid (89%) not enhance this process. It was suggested that further removal of phytate did not improve the digestibility of protein.

Table 4

Retention of phytic acid in soybean flour (mg/g) after soaking at 30˚C for different times with 2% sodium bicarbonate ($NaHCO_3$). Before soaking phytic acid level in soybean was in V1 (12mg/g), V2 (11 mg/g), V3 (13 mg/g) and V4 (12.5 mg/g)

Variety	Time(h)	Phytic acid (mg/g) Soybean flour			Mean
		Water	PH 4	pH 6	
V1	6	8.45C	7.28H	6.67M	7.47C
	12	6.66N	6.30P	5.65V	6.20G
V2	6	7.68E	6.68L	6.12R	6.83D
	12	5.96T	5.58W	5.10X	5.55H
V3	6	9.18A	7.92D	7.27I	8.12A
	12	7.35G	6.58O	6.07S	6.68E
V4	6	8.86B	7.56F	6.90J	7.78B
	12	6.80K	6.18Q	5.84U	6.27F
Mean		7.617A	6.760B	6.203C	

Figures showing the same letter(s) are statistically not different from one another (LSD Test).

Table 5

Retention of phytic acid in soybean flour (mg/g) after soaking at 30˚C for different times with 1% sodium chloride (NaCl) + 2% sodium bicarbonate ($NaHCO_3$). Before soaking phytic acid level in soybean was in V1 (12 mg/g), V2 (11 mg/g), V3 (13 mg/g) and V4 (12.5 mg/g)

Variety	Time(h)	Phytic acid (mg/g) soybean flour			Mean
		Water	pH 4	pH 6	
V1	6	7.48	5.72	5.28	6.16B
	12	6.20	5.48	4.12	5.27CD
V2	6	6.80	5.33	4.39	5.51C
	12	5.81	5.05	3.60	5.15D
V3	6	7.92	6.21	5.70	6.61A
	12	6.71	5.82	4.28	5.60C
V4	6	7.80	6.08	5.56	6.48AB
	12	6.65	5.57	4.22	5.48CD
Mean		6.921A	5.657B	4.643C	

Figures showing the same letter(s) are statistically not different from one another (LSD Test).

Table 6
Effect of food additives on average percent reduction of phytic acid of four varieties of soybean soaked in water and solutions of pH4 and pH 6 for 6 h

Food additive used	Phytic acid (% reduction)		
	Water	pH 4	pH 6
No additive	8	6	12
1% NaCl	8	11	16
2% $NaHCO_3$	40	40	45
1% NaCl + 2% $NaHCO_3$	38	52	56

Table 7
Effect of food additives on average percent reduction of phytic acid of four varieties of soybean soaked in water and solutions of pH 4 and pH 6 for 12 h

Food additive used	Phytic acid (% reduction)		
	Water	pH 4	pH 6
No additive	7	10	18
1% NaCl	10	14	21
2% $NaHCO_3$	45	50	53
1% NaCl + 2% $NaHCO_3$	48	55	68

The effect of soaking on phytic acid content in soybean in tap water, pH 4 and pH 6 solutions in the presence of 2% $NaHCO_3$ at 30˚C for 6 and 12 h is shown in Table 4 and analysis of variance is given in Appendix-III. Soaking for 6 h, in V1 of soybean, the phytic acid was reduced from its original level (12 mg/g) in water, pH 4 and pH 6 solutions to 8.45 mg, 7.28 mg and 6.67 mg/g, and in V2 (11 mg/g) to 7.68 mg, 6.68 mg and 6.12 mg/g, respectively. Similarly in V3, the phytic acid (13 mg/g) was reduced to 9.18 mg, 7.92 mg and 7.27 mg/g and in V4 (12.50 mg/g) to 8.86 mg, 7.56 mg and 6.90 mg/g respectively. Soaking the soybean for 12 hours at 30˚C, in tap water, pH 4 and pH 6 solutions the phytic acid in V1 was reduced to 6.66 mg, 6.30 mg and 5.65 mg/g and in V2 phytic acid reduced to 5.96 mg, 5.58 mg and 5.10 mg/g respectively. In V3, the phytic acid reduced to 7.35 mg, 6.58 mg and 6.07 mg/g and in V4 phytic acid reduced to 6.80 mg, 6.18 mg and 5.84 mg/g respectively. In this treatment the effect of pH6 for 12 h was most effective in lowering the phytic acid in soybean. Statistically, the results show that soaking in tap water, pH4 and pH6 solutions in the presence of 2% $NaHCO_3$ for both times 6 and 12 h significantly reduced ($P < 0.05$) the level of phytic acid in all four varieties of soybean.

The results in Table 5 show the effect of soaking on phytic acid content in soybean in tap water, pH 4 and pH 6 solutions in the presence of 1% sodium chloride (NaCl) + 2% sodium bicarbonate ($NaHCO_3$) at 30˚C for 6 and 12 h. The analysis of variance is given in Appendix-IV. Soaking for 6 hours, in V1 of soybean the phytic acid was reduced from its original level (12 mg/g) in water, pH4 and pH6 solutions to 7.48 mg, 5.72 mg and 5.28 mg/g and in V2 (11 mg/g) to 6.80 mg, 5.33 mg and 4.39 mg/g, respectively. Similarly in V3 the phytic acid (13 mg/g) was reduced to 7.92 mg, 6.21 mg and 5.70 mg/g and in V4 (12.50 mg/g) to 7.80 mg, 6.08 mg and 5.56 mg/g, respectively. Soaking soybean for 12 h at 30˚C in tap water, pH 4 and pH 6 solutions the phytic acid in V1 was reduced to 6.20 mg, 5.48 mg and 4.12 mg/g and in V2 to 5.81 mg, 5.05 mg and 3.60 mg/g, respectively. In V3, the phytic acid reduced to 6.71 mg, 5.82 mg and 4.28 mg/g and in V4 to 6.65 mg, 5.57 mg and 4.22 mg/g respectively (Table 6 & 7).

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