PHYTATE CONTENT IN FERMENTED SOYBEANS
KANDUNGAN FITAT PADA KEDELAI YANG DIFERMENTASI
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ABSTRACT

The content of phytate in soybeans meal fermented by lactic acid producing bacteria (LAPB) and by Rhizopus oligosporus in tempeh was estimated by trimetric chloride precipitation method and anion exchange chromatography procedures.

Phytate determination by the anion exchange chromatography method provided more precise values than ferric chloride precipitation method. Estimation of phytate content by these two method showed that fermentation, using lactic acid producing microorganism significantly decreased (P < 0.05) phytate content both in subsoaked and boiled soybeans.

Incubation of tempeh for 24 hours at 30°C increased the phytate content from 1.62% in boiled soybean to 1.64% in tempeh. These values were also estimated by anion exchange chromatography.

Keyword: phytate - soybean - fermentation.

Abstrak

Kandungan asam fitat dalam kedelai yang difermentasi dengan bakteri penghasil asam laktat dan dalam tempeh yang menggunakan Rhizopus oligosporus diukur dengan metode pengendapan fenilhidrata dan kromatografi pertukaran anion.

Penentuan dengan kromatografi pertukaran anion memberikan hasil yang lebih baik (standard deviasi lebih kecil) dibanding dengan cara pengendapan fenilhidrata.

Hasil dari kedua cara menunjukkan bahwa kedua proses fermentasi tersebut dapat menurunkan kadar fitat dalam kedelai baik yang direbus maupun yang diasuki. Kata kunci: asam fitat - kedelai - fermentasi.

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Introduction

Phytate is found almost exclusively in plants, primarily in the seeds of cereal grains and oilseeds (O’Dee et al., 1972). Phytate represents 60 to 80% of the total organic phosphorus of cereal grains (Lolas et al., 1976; Erdman, 1979). Phytic acid functions as a storage form of phosphorus during germination (Asada, 1969).

Phytate content in soybean seeds varies according to the variety (Lolas et al., 1976), while in soybean products, the phytic acid content varies depending on the method of processing. Phytic acid is the hexa-phosphate of myoinositol and has three strongly bound water molecules. Early studies reported the structure of phytate as "inositol hexaphosphate". Due to the large number of acidic groups the phytate molecule has the property of forming a variety of salts. It can form a number of insoluble salts with different metal ions and can sequester several metals by chelate formation.

Phytate also complexes with protein making the less soluble (Lolas & Markakis, 1975; O’Dell and de Boland, 1976; Erdman and Forbes, 1981; Jaffe, 1981). Barre (1956) (cited by O’Dell and de Boland, 1976) reported that phytate-protein complexes are less subject to proteolytic digestion than the same protein alone.

In soybeans, which phytate is concentrated within protein bodies, disruption of the soybean cotyledon makes it possible for phytic acid to react strongly with glycine, the major protein body (Tombs, 1967). Depending upon pH, ionic strength and other conditions, phytic acid can interact chemically with other components of the soybean (Erdman and Forbes, 1981).

Rakis and Anderson (1977) postulated that the formation of a protein-phytate-mineral complex during manufacture, is the primary factor responsible for the reduction of availability of mineral in some soy products. However, the action of phytase to degrade phytic acid, or the removal of phytic acid, increase bioavailability of mineral in some soy products. Lopez et al. (1983) observed that natural lactic acid fermentation decrease the phytic acid in cow's milk due to phytases elaborated by microorganisms. Also, strong phytase activity was demonstrated by Rhizopus oligosporus when use in tempeh fermentation (Sudarmaji and Markakis, 1977). It appears that fermentation has a potential for decreasing the phytate content of soybean meal, thus increasing the bioavailability of mineral. Therefore the objective of this study was to evaluate the phytic acid content before and after fermentation in soybeans.
Materials and Method

Preparation of samples

The source of sample, cleaning procedures, heat treatments, procedures for fermentation with LAPB and fermentation with R. oligosporus were described by Moeljopawiro et al. (1987).

Determination of phytate

Two methods were used to determine the phytate content of samples: ferric chloride precipitation and anion exchange chromatography. The phytate was calculated on the basis of a 28.2% phosphorus content.

1. Ferric chloride precipitation procedure.

A modification of the ferric chloride precipitation procedures described by de Boland (1970) and Ellis et al. (1977) was used to determine phytate content. The extraction solution was made by placing 32.4 ml of hydrochloric acid and 100 g of sodium sulfate into a 1-L volumetric flask and bringing to volume with distilled water. Hydrochloric acid 0.5 N was used as the washing solution. The phytate was precipitated with 0.4% ferric chloride instead of 1.2% ferric chloride as used by de Boland (1970). It was found that a 0.4% ferric chloride solution was effective in allowing the formation of ferric phytate salt. The 0.4% ferric chloride was prepared by dissolving 6.7 g of ferric chloride (FeCl₃·6H₂O) and 50 g of sodium sulfate in 300 ml of double distilled water and combining with 16.2 ml of hydrochloric acid (12 N) after which the solution was brought to a volume of 1000 ml with double distilled water.

One g of sample was placed in a 250 ml Erlenmeyer flask with a teflon screw cap. Fifty ml of extraction solution were added, and the sample was shaken overnight at 20°C using a shaking water bath (GCA Corporation, Precision Scientific Group, Chicago, IL). After the sample was removed from the shaker, it was filtered under vacuum through Whatman No.1 filter paper with a Buchner funnel. Ten ml of filtrate was quantitatively transferred into a 50 ml centrifuge tube. Ten ml of double distilled water and 5 ml of precipitation solution were added. The tube was placed in a boiling water bath for 15 minutes and then was transferred immediately to an ice
bath for 5 minutes. The precipitate and solute were separated by centrifugation for 10 minutes at 3,000 rpm. After the solute was removed, the precipitate was resuspended in 5 ml of wash solution for 10 minutes and recentrifuged. The washing procedure was performed three times. Three ml of concentrated nitric acid and 1.0 ml of concentrated sulfuric acid were added to the washed sample which was then transferred quantitatively into a 100 ml Kjeldahl flask. The sample was digested using a Lab Con Co Kjeldahl digestion unit until the solution was clear. After the sample was cooled, double distilled water was added (10 ml), and it was then brought to boil to ensure the hydrolysis of pyrophosphate compounds. The sample was allowed to cool at room temperature. It was then transferred to a 50 ml volumetric flask and brought to volume with double distilled water for analysis of phosphorus. The determination of phosphorus were done by the procedure of Fiske and Subbarow (1925).

2. Ion exchange chromatography procedure.

The phytate was extracted from the sample using 2.4% hydrochloric acid. A 0.7 M sodium chloride was used to elute phytate from the column. The lower ionic strength used for the first elution was 0.1 M sodium chloride.

(a) Column preparation.

Three ml of double distilled water was added to a glass barrel column (0.7 x 15 cm), and the slurry of 0.5 g resin (anion exchange resin, AG 1-X4, 100-200 mesh, chloride form, Biorad Laboratories, Richmond, CA) was poured into the column. After the resin bed was formed, the column was washed, first, with 3 volumes of 0.7 M sodium chloride and then with 3 volumes of double distilled water.

(b) Blank preparation.

One ml of 2.4% hydrochloric acid was transferred into a 25 ml volumetric flask, 1.0 ml of Na₂EDTA-NaOH was added, and the solution was brought to volume with double distilled water and shaken well. The Na₂EDTA-NaOH solution was made by placing 10.23 g disodium ethylene-diaminetetraacetate (Na₂EDTA) and 7.5 g NaOH in a 250 ml volumetric flask and bringing to volume with double distilled water.
(c) Standard curve preparation.

Into each of three 50 ml volumetric flasks, 0.5, 1.0, and 1.5 ml of standard phosphate solution containing 80 micrograms of phosphorus per ml (NERL-East Providence, RI) were added. Then, 20 ml of double distilled water was added, and the solution was mixed. Two ml of 2.4% ammonium molybdate in 1.0 N sulfuric acid (12.5 g ammonium molybdate, 50 ml of 10 N sulfuric acid, and double distilled water to bring to a final volume of 200 ml) were added and mixed well. Then 1.0 ml of sulfonic acid solution was poured into the flask and mixed well. It was brought to volume with double distilled water and shaken thoroughly. After 15 minutes the absorbance was read at 640 nm on a Spectronic-21 spectrophotometer.

(d) Sample assay.

Two g of sample were placed in a 125 ml Erlenmeyer flask with a teflon screw cap, and 40 ml of 2.4% hydrochloric acid were added. The sample was then placed in a shaking water bath at room temperature (20°C) and shaken for 3 hours. After 3 hours, the sample was filtered with vacuum through Whatman No. 1 filter paper. One ml of filtrate was transferred quantitatively into a 25 ml volumetric flask, and 1.0 ml of Na₂EDTA-NaOH solution was added. It was brought to volume with double distilled water and mixed well. The solution was then poured quantitatively onto the prepares column, and the eluate was discarded. The column was washed with one volume of double distilled water, followed by pouring 15 ml of 0.1 ml NaCl on the column and discarding the eluate. The phytate was eluted with 15 ml of 0.7 M NaCl, and the fraction was collected in a 100 ml Kjeldahl flask. Three ml of concentrated nitric acid and 0.5 ml concentrated sulfuric acid were added to the flask. The mixture was digested using a Lab Con Co Kjeldahl digestion unit over medium heat. After the solution was clear, the heat was reduced to low for 5 minutes. The flask was cooled, 10 ml of double distilled water were added and swirled to dissolve the salt, and it was reheated for 10 minutes. The solution was allowed to cool before transferring it quantitatively into a 50 ml volumetric flask. Two ml of ammonium molybdate solution were added and mixed well, then 1.0 ml of sulfonic acid reagent was added and mixed. The solution was brought to volume with double distilled water and allowed to stand for 15 minutes, after which absorbance was read at 640 nm on a Spectronic-21 spectrophotometer.
(c) Calculation.

The phytate was calculated on the basis of a 28.2% phosphorus content. Phytate content of the sample was calculated using the following formula:

\[ \text{Mean K} \times \text{Absorbance} \times 20 \times \frac{\text{mg phytate}}{0.282 \times 1.000} = \frac{\text{g sample}}{\text{g sample}} \]

"K" was derived by dividing the concentration of phosphorus in a standard by the absorbance obtained from the standard. It represents the calculated concentration per unit absorbance for each concentration of standard. The "Mean K" was obtained from the average of several K values of a series of standards with different phosphorus concentrations. Twenty is a dilution factor derived as follows: 40/2 = 20, where 40 was mls of extraction solution, 2 was the weight of the sample in g, and 1.0 ml of extract was placed on the column. Data of the phytate content were presented on the dry basis. The moisture content of the samples were determined using the vacuum oven (AOAC, 1980).

Statistical Analysis

In reporting the results of this investigation only statistically significant differences at \( p<0.05 \) are discussed. Analysis of variance, one way classification was used to evaluate the content of phytate in the samples.

Results and Discussion

The phytate content of soybean products was determined by two methods: ferric chloride precipitation and anion exchange chromatography. Values (except those for raw soybeans) obtained by the anion-exchange chromatography method were higher than those by the precipitation method (Table 1). The difference may be due to the loss of phytate in the washing process employed in the precipitation method. When the standard deviations for the two methods are compared, it appears that the anion exchange chromatography method provided more precise values than the ferric chloride precipitation method (Table 1). The anion exchange resin resulted in a specific separation of the phytate anion. Inorganic phosphorus was eluted through the column with water and 0.1 M NaCl, and phytate phosphorus was eluted with 0.7 M NaCl.

The author agrees with Harland and Oberleas (1977) that the anion exchange chromatography method is simple, reproducible, and rapid.
Thompson and Erdman (1982) postulated that the anion-exchange chromatography method was most accurate but not convenient for routine analysis.

1. Raw soybeans.

The mean phytate content of the Williams variety of soybeans that were used in this study was 1.06% by the ion-exchange chromatography procedure (Table 1). Lolas et al. (1976)

Table 1. Means of phytate content in soybean products.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Precipitation</th>
<th>Ion exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>1.15± 0.04</td>
<td>1.06± 0.006</td>
</tr>
<tr>
<td>Autoclaved non-fermented</td>
<td>1.23± 0.04</td>
<td>1.38± 0.03</td>
</tr>
<tr>
<td>Boiled non-fermented</td>
<td>1.42± 0.08</td>
<td>1.62± 0.006</td>
</tr>
<tr>
<td>Autoclaved fermented</td>
<td>1.07± 0.02</td>
<td>1.24± 0.006</td>
</tr>
<tr>
<td>(pH 6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoclaved fermented</td>
<td>1.18± 0.07</td>
<td>1.26± 0.006</td>
</tr>
<tr>
<td>(pH 4.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled fermented</td>
<td>1.24± 0.06</td>
<td>1.29± 0.02</td>
</tr>
<tr>
<td>(pH 6.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled fermented</td>
<td>1.31± 0.07</td>
<td>1.37± 0.03</td>
</tr>
<tr>
<td>(pH 4.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tempeh</td>
<td>1.36± 0.06</td>
<td>1.44± 0.01</td>
</tr>
</tbody>
</table>

\(^1\) Where letters differ within a column, means differ significantly (P<0.05) from each other.
observed that phytate content in soybeans varied depending on the variety. From among 15 varieties, the lowest phytate content was 1.00% for the Kim IA variety, and the highest was 1.47% for the 71-6234 S.R.L. variety.

2. Autoclaved soybeans.
A decrease in phytate content caused by autoclaving was not observed in this study (Table 1). This is not consistent with the findings of de Bolard (1970) that autoclaving at 120°C for 4 hours reduced the phytate content in soybean flakes to 72.9% of its original content. The phytate content in soybean protein after autoclaving under the same conditions was reduced to 41.1%. Rashbora et al. (1974) pointed out that the phytate content in processed soybeans varies depending on the method of processing. When cooking phytate-containing food, temperature and time do not appear to affect the phytate breakdown (Oberleas, 1983).

The mean phytate content of boiled non-fermented soybeans (1.62%) was higher than the 1.06% of whole raw soybeans (Table 1). This finding was in agreement with results of Sutardi and Buckle (1985), that the phytate content of raw dried soybeans, soybeans soaked for 24 hours and soybeans soaked (24 hours) followed by boiling for 5 minutes was 1.07%, 1.69% and 1.68%, respectively.

The high phytate content of boiled, soaked soybeans is related either to the loss of solids during soaking and boiling or to phenomena occurring during the soaking process. Loss of solids leads to an increase in the concentration of phytate. Soaking of dried soybean initiates the germination process, and this may account for some increase in phytate content. Mayaner et al. (1972) showed that phosphoinositol kinase activity increased during germination of mung bean seeds. These enzymes mediate the phosphorylation of lower inositol phosphate to their higher homologous forms with ATP as a phosphate donor and may contribute to an increase in the level of phytate.

Lasas and Markakis (1977) found that phytic acid in Navy beans remained constant after soaking for 24-48 hours, whereas Tabekhia and Lush (1980) observed that, in four other bean varieties, soaking for 12 hours decreased phytate content between 9 and 19%. Soaking followed by cooking under pressure decreased the phytate content of peas by 13% (Becal and Moltza, 1985). In view of the apparent lack of agreement among the various researchers, further studies are needed to elucidate the influence of soaking and boiling on the phytate content of soybeans.

82
4. Lactic acid fermented soybeans

The phytate content of lactic acid fermented soybeans, both autoclaved and boiled, was lower than that of the non-fermented products (Table 1). The reduction of phytate in the fermented soybeans was due to the action of the phytase enzymes produced by lactic acid microorganisms. Lopez (1982) reported that 80% of the microorganisms isolated from lactic acid fermented corn elaborated the phytase enzyme.

Adjustment of the pH to original pH after fermentation did not affect the content of phytate. The phytate content of acid products (pH 4.2) did not differ from that of the neutral products (pH 6.5 or 6.7) (Table 1). This was consistent with data presented by Erdman et al, (1980) which indicated that the phytate content in acid soy isolates (pH 4.5) was 1.52% and did not differ from that found in neutral soy isolates (pH 7.0).

Conclusion

Phytate in autoclaved soybeans and boiled soybeans can be decreased by fermentation either by LAPB or by Rhizopus oligosporus.

Determination of phytate by anion exchange chromatography method provided more precise values than the ferric chloride precipitation method.

REFERENCES


84