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Full Length Research Paper

Optimization of optimum condition for phytic acid extraction from rice bran

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Phytic acid is one of the bioactive compounds that are being intensively studied to evaluate their effects on health. This study was carried out to determine the optimum condition for phytic acid extraction from rice bran. Three main parameters were considered to optimize the condition for phytic acid extraction; different types of extracting solvent, length of extraction time and influence of different pH adjustment. Three acidic solutions were selected and were trichloroacetic acid (TCA), hydrochloric acid (HCl) and sulphuric acid (H_2SO_4). The results showed that 5% of H_2SO_4 in pH 0.6 and after 30 min of extraction time gave the highest amount of phytic acid compared to 10% of TCA and 3% of HCl. The content of phytic acid in rice bran ranged from 0.22 to 2.22% for the different parameters optimized. Different methods produced different content of phytic acid from rice bran. Therefore, 5% of H_2SO_4 in pH 0.6 and 30 min of extraction time was the best condition for the optimum production of phytic acid.

Key words: Phytic acid, rice bran, extraction, optimization, purification, HPLC.

INTRODUCTION

Phytic acid is one of the bioactive compounds that are being intensively studied to evaluate their effects on health. It has been shown to have potential as anticancer agent which only affects malignant cells and does not affect normal cells and tissues (Vucenik and Shamsuddin, 2003). Phytic acid is a simple ranged carbohydrate with six phosphate groups attached to each carbon (Shamsuddin, 2002). It serves as the major phosphorus storage compound in plant in the seed, as well as being a natural antioxidant by its chelating properties and reduction of the catalytic activities of many divalent transition metals (Verghese et al., 2006). The chelation ability of phytic acid with minerals has been suggested to have beneficial effects toward lowering serum cholesterol and triglycerides and suppression of iron-mediated oxidation (Lee et al., 2005). A variety of benefits of phytic acid on human health have also been reported including its potential as an anti-cancer properties in soft tissue, colon, prostate, metastatic and mammary cancers. It may also act as an inhibitor for renal stone development (Dost and Tokul, 2006).

In whole grain cereals such as corn, wheat and rice, the ranges of phytic acid is from 1.5 to 6.4% while defatted and dehulled oilseed meals such as soy, peanut and sesame contain 1.5% or more of the compound (Grases et al., 2004). Phytic acid is primarily found in the outer layers of rice bran. Rice bran is a by-product of rice milling to produce white rice to fulfill its desirability. Malaysia is one of the world's rice producing and consuming country where the consumption of rice in Malaysia alone increases every year and can reach as much as 1.96 million tones of paddy by year 2010 (Hossain and Narciso, 2004). Recent studies demonstrated that the antinutrient effect of phytic acid can be manifested only when large quantities of phytic acid are consumed in combination with a diet poor in oligoelements (Shamsuddin and Vucenik, 2005). Several methods are available for determining the phytic acid concentrations in cereal products, biological and urine

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samples. These methods are liquid chromatography-conductimetric detection with ion suppression and refractive index detection and ultraviolet (UV) detection, liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry and inductively coupled plasma atomic emission spectrometry. Other alternatives to determine the phytic acid concentration involve its hydrolysis and the determination of the hydrolysis products, such as phosphate or inositol. The existence of phosphate can be determined spectrophotometrically whereby the inositol can be determined by gas chromatography (GC) after derivation (Dost and Tokul, 2006). Several derivatization reagents have also been proposed such as trimethylchloroacetic anhydride (March et al., 2001). In this study, the optimization of the condition for the extraction of phytic acid from defatted rice bran was focused on using spectrophotometrical method which is simple and rapid. Three main parameters that were considered are the different type of extracting solvent, length of the extraction time and the influence of different pH adjustment on the total phytic acid content.

MATERIALS AND METHODS

Plant material

Freshly milled raw rice bran samples from mixed local varieties were obtained from local supplier. The raw rice bran was transported in dry ice in cold box containers. Immediately after it reached the laboratory, the rice bran samples were passed through a 600 µm sieve to achieve appropriate particle size.

Chemicals

The chemicals used included hexane (Merck, Germany), trichloroacetic acid (Appli Chem, German), hydrochloric acid (BDH Chemicals, England, United Kingdom), sulphuric acid (BDH Chemicals, England, United Kingdom) and Iron (III) Chloride (Sigma, St. Louis, MO, USA).

Instruments and equipments

Ultracentrifuge (Beckman TL 100 Table Top Ultracentrifuge – Palo, Alto, CA), spectrophotometer (Secomam CE, France), orbital mixer (Thermolyne Co., USA), Shaker (Protech, Malaysia), high performance liquid chromatography (HPLC) (Agilent 1100 series G1311A), Column C_{18} Bondapac (250 cm × 4.6 mm), and electronic balance (Fy-350, A&D Company, Ltd, Japan) were used for the analyses.

Stabilization of rice bran

Rice bran was stabilized according to the method of Ramezanzadeh et al. (2000). Stabilization was performed to prevent oxidative rancidity during storage. It was then microwaved at 2450 MHz. For each time, 100 g of bran was stabilized for 1 min. Then, the sample was mixed homogenously and microwaved again for another 1 min. The sample was allowed to cool at room temperature before it was put in dark plastic bags and stored at 20°C till use.

Lipid extraction

Total lipid was extracted from rice bran samples by using hexane, based on the modified method of Zullaikah et al. (2009). 2 ml of hexane was added to 0.5 g of rice bran and soaked overnight. This was followed by evaporation of rice bran to dryness using vacuum pump apparatus. The samples then were stored at 20°C until further analysis.

Standard curve preparation

The method used was adapted from Haug and Lantzch (1983). 0.15 g of sodium salt of phytic acid, obtained from Sigma (No. P8810) was diluted into 100 ml of distilled water. The solutions were prepared by diluting the stock in a range from 3-30 µg/ml phytate phosphorus which contained about 1.2-11.7 ml stock solution in 100 ml.

Phytic acid extraction from rice bran

Extraction of phytic acid from defatted rice bran was based on the procedures of Fruhbeck et al. (1995) with some modification. The samples were added to acidic solution of trichloroacetic acid (TCA), hydrochloric acid (HCl) and sulphuric acid (H_2SO_4) in selected concentrations and the appropriate pH was adjusted. The extraction was carried out at room temperature and constant shaking at medium speed in an orbital mixer in different length of extraction time. The obtained creamy mixture was centrifuged at 17,300 rpm for 30 min at 15°C and the supernatants were collected.

Determination of phytic acid

Phytic acid content in the extract was quantified by using spectrophotometrical method (Haug and Lantzh, 1983; Butt et al., 2004). In this procedure, the decrease in iron (determined calorimetrically with 2,2'-bipyridine) in the supernatant was measured; the content of phytic acid. 1 ml of ferric (III) chloride solution was added to 0.5 ml extract. The solution was heated for 30 min in a boiling water bath. After being cooled to room temperature, the solution was centrifuged for 30 min at 4500 rpm. Then, 1 ml of the supernatant was transferred to another test tube and mixed with 2,2'-bipyridine. The absorbance of the reaction mixture was measured at 519 nm against distilled water. The method was calibrated with standard phytic acid solutions for each set of analysis.

Statistical analysis

All the measurements were duplicated on triplicate samples (3 x 3). The results were statistically analyzed by the analysis of variance (ANOVA) and Turkey test. Statistical significance was accepted at level of $p<0.05$.

RESULTS

A variety of methods were tested throughout the twentieth century to attain precise identification of phytic

Table 1. Phytic acid content (%) of rice bran by using TCA as extracting solvent

Acidic solution (TCA) (%)	pH	Extraction time (h)	Phytic acid
3	0.2	2	1.07
	0.2	3	1.24
	0.6	2	1.16
	0.6	3	1.51
	1.0	2	1.44
	1.0	3	1.65
5	0.2	2	1.41
	0.2	3	0.67
	0.6	2	1.27
	0.6	3	1.46
	1.0	2	1.49
	1.0	3	1.74
10	0.2	2	1.81
	0.2	3	0.77
	0.6	2	1.69
	0.6	3	1.26
	1.0	2	1.68
	1.0	3	1.77

Table 2. Phytic acid content (%) of rice bran by using H_2SO_4 as extracting solvent.

Acidic solution (H_2SO_4) (%)	pH	Extraction time (h)	Phytic acid (%)
3	0.6	0.5	1.70
	0.6	1	1.72
	0.8	0.5	1.80
	0.8	1	1.77
5	0.6	0.5	2.22
	0.6	1	1.76
	0.8	0.5	1.90
	0.8	1	1.92
10	0.6	0.5	1.82
	0.6	1	1.96
	0.8	0.5	2.04
	0.8	1	1.86

acid. However, there is no known specific reagent for the identification of phytic acid. In this study, three main parameters were considered to optimize the best condition to extract phytic acid from rice bran. These were the different type of extracting solvent, length of the extraction time and the influence of different pH adjustment on total phytic acid content. Tables 1, 2 and 3 present the content of phytic acid obtained by using different extracting solvents, length of extraction time and of different pH adjustment. Table 4 summarizes the results of phytic acid content in the three different extracting solvents obtained by the optimization of different conditions of extraction. The calibration curve of phytic acid is shown in Figure 1. A linear standard curve was obtained by plotting the decrease in absorbance at 519 nm against phytate concentration. The range for analysis was from 3 to 30 µg/ml phytate phosphorus. The value of the correlation coefficient (r=-0.9867) of the calibration curve represented a strong negative relationship between absorbance and concentration of phytate phosphorus. As the value of the absorbance increased, the amount of phytate phosphorus decreased. The total content of phytic acid in rice bran was determined using the linear equation, $Y= - 0.0037x + 1.1396$. Phytic acid was analyzed by reverse-phase high performance liquid chromatography (HPLC). HPLC chromatograms were compared with standard phytic acid, in which two peaks were determined as shown in Figure 2. The first peak represented phytic acid and the second peak represented inositol. Standard phytic acid was diluted into four concentrations; 50, 100, and 1000 ug/ml. The sample phytic acid was also diluted into four concentrations; 50, 100, and 1000 ug/ml. The two peaks that were obtained in this study were similar to those obtained in the standard phytic acid. The retention time for phytic acid extracted from rice bran was 4.167 and the retention obtained in the standard phytic acid was 3.461.

DISCUSSION

Extraction is the process of dissociation and acid solubilization of phytate from its native matrix whether it is a mixed salt or associated with protein. The essential aspect of extraction is to recover as much phytate from the sample matrices with the conditions used. The first parameter that was considered was type and the concentration of extracting solvent. The three acidic solutions that were selected were trichloroacetic acid (TCA) (Xu et al., 1992), hydrochloric acid (HCl) (Fruhbeck et al., 1995) and sulphuric acid (H_2SO_4) (Xu et al., 1992). Several acidic solutions, in various concentrations were selected according to the conditions that may be appropriate for a specific group of foodstuffs. It was based on the principal that phytate was the only phosphate compound that forms an insoluble complex with ferric iron in dilute acid (Oberleas and Harland, 2001). For TCA as an extracting solvent, three different concentrations were used (3, 5 and 10). Three different concentrations were selected when HCl was utilized as an extracting solvent which are 1.5, 3 and 3.7% and for sulphuric acid (H_2SO_4), the concentrations utilized were 3, 5 and 10%. The

Table 3. Phytic acid content (%) of rice bran by using HCl as extracting solvent.

Acidic solution (HCl) (%)	pH	Extraction time (h)	Phytic acid (%)
1.5	0.2	2	0.23
	0.2	3	1.18
	0.6	2	1.37
	0.6	3	1.69
	1.0	2	1.65
	1.0	3	1.42
3.0	0.2	2	1.90
	0.2	3	0.41
	0.6	2	1.73
	0.6	3	1.81
	1.0	2	0.72
	1.0	3	2.08
3.7	0.2	2	0.22
	0.2	3	1.84
	0.6	2	1.38
	0.6	3	0.82
	1.0	2	1.63
	1.0	3	

Table 4. Summary of the highest phytic acid content (%) of rice bran in the three different extracting solvent by optimization of different condition of extraction.

Extracting solvent	pH	Extraction time (h)	Phytic acid (%)
10% TCA	1.0	3	1.77[a]
5% H_2SO_4	0.6	0.5	2.22[b]
3% HCl	1.0	3	2.08[c]

Each value represents three replications ± SD. Values with different letter indicates significant difference ($P<0.05$).

selection of concentration was done according to the previous studies that were conducted for phytic acid extraction (Xu et al., 1992; Fruhbeck et al., 1995). The results in Table 4 also showed that there was a significant difference between the amount of phytic acid obtained by using 10% of TCA, 5% of H_2SO_4 and 0.8 M of HCl. The content of phytic acid obtained in this result was approximately in line with the finding reported by Wei et al. (2007). However, the content of phytic acid obtained in this finding was lower than that attained in rice bran from the reports of Ravindran et al. (1994) and Knuckles et al. (1982). This may be due to the different methods and condition applied; solvents, pH and time used to extract phytic acid. The studied conducted by Liu et al. (2005) reported that phytic acid content in japonica rice cultivars ranged from 0.685 to 1.03% which was collected from different areas of China while, De Boland et al. (1975) reported that brown rice contained 0.89% of phytic acid, whereas the germ had 3.48% and the pericarp 3.37%.

The results also showed that 5% of H_2SO_4 gave the highest amount of phytic acid as compared to 10% of TCA and 0.8 M of HCl. This finding is consistent with that of Camire and Clydesdale (1982) which reported that H_2SO_4 gave the highest content of phytic acid in wheat bran. They also reported that 3% of TCA was more effective than 3% of H_2SO_4 in the extraction of phytic acid from wheat protein concentrate. Cilliers and Niekerk (1986) reported that 3% of TCA was the preferred extracting solvent for phytic acid extraction. Boss et al. (1992) reported that extraction with HCl provided consistently higher extraction rates. The second factor that was considered to have influences on the optimization of the best condition for phytic acid extraction from rice bran was pH adjustment. According to Gifford and Clydesdale (1990), the low pH value between 0 and 1 is needed to dissociate phytate from iron and protein complexes. Consequently, different pH adjustments were considered in the extraction step. In TCA and HCl, the pHs were adjusted to pH 0.2, 0.6 and 1.0 respectively, whereas for H_2SO_4, the acidic solution were adjusted to pH 0.6 and 0.8. The results obtained showed that by adjusting 5% of H_2SO_4 to pH 0.6, the highest amount of phytic acid level was assessed. This finding is consistent with the report by Fruhbeck et al. (1995) which reported that when pH was adjusted to pH 0.6 in the extraction step, phytate assessment was Length of time was another significant consideration for phytic acid extraction. The shortest extraction time reported was 5 min blending of potato or other plant tissue with 100 ml in a waring blender (Oberleas and Harland, 1986). According to Oberleas and Harland (2001), ideally 2 h is the minimum extraction time that can be recommended and 3 h may be optimum. However, according to the report by Lehrfeld (1989), when finely comminuted samples are subjected to ultrasonic treatment, extraction times at room temperature ranges from 0.5 to 2 h with vigorous agitation which is reduced to 1-3 min. In this study, different ranges of extraction time were selected. By using trichloroacetic acid and hydrochloric acid, 2 and 3 h were used respectively for the extraction of phytic acid, and for sulphuric acid, 1 h and 30 min were used to extract phytic acid. The results in Table 4 shows that 30 min of extraction time with 5% of H2SO4 as extracting reagent gave the optimum time for phytic acid extraction in rice bran. For TCA and HCl, 3 h of extraction time gave the highest amount of phytic acid. The length of extraction time used were similar as used by Averill and King (1986) that analyzed a large number of foodstuffs using 3 h extraction period for their analyses. Studies by Tangendjaja

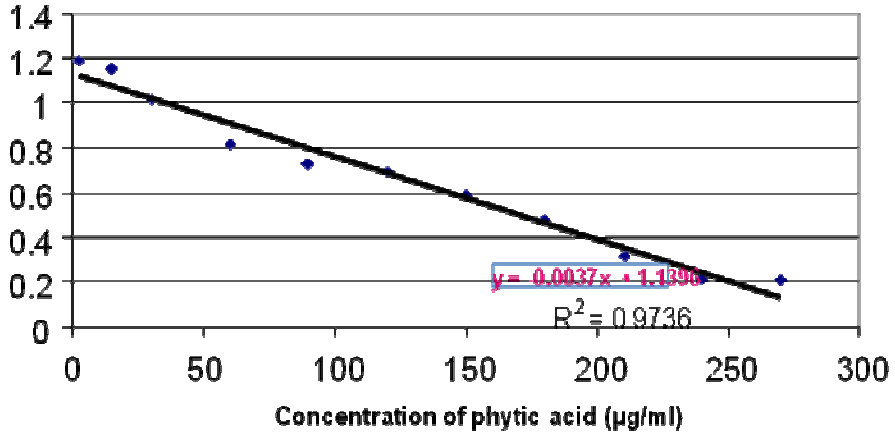

Figure 1. Calibration curve of phytic acid.

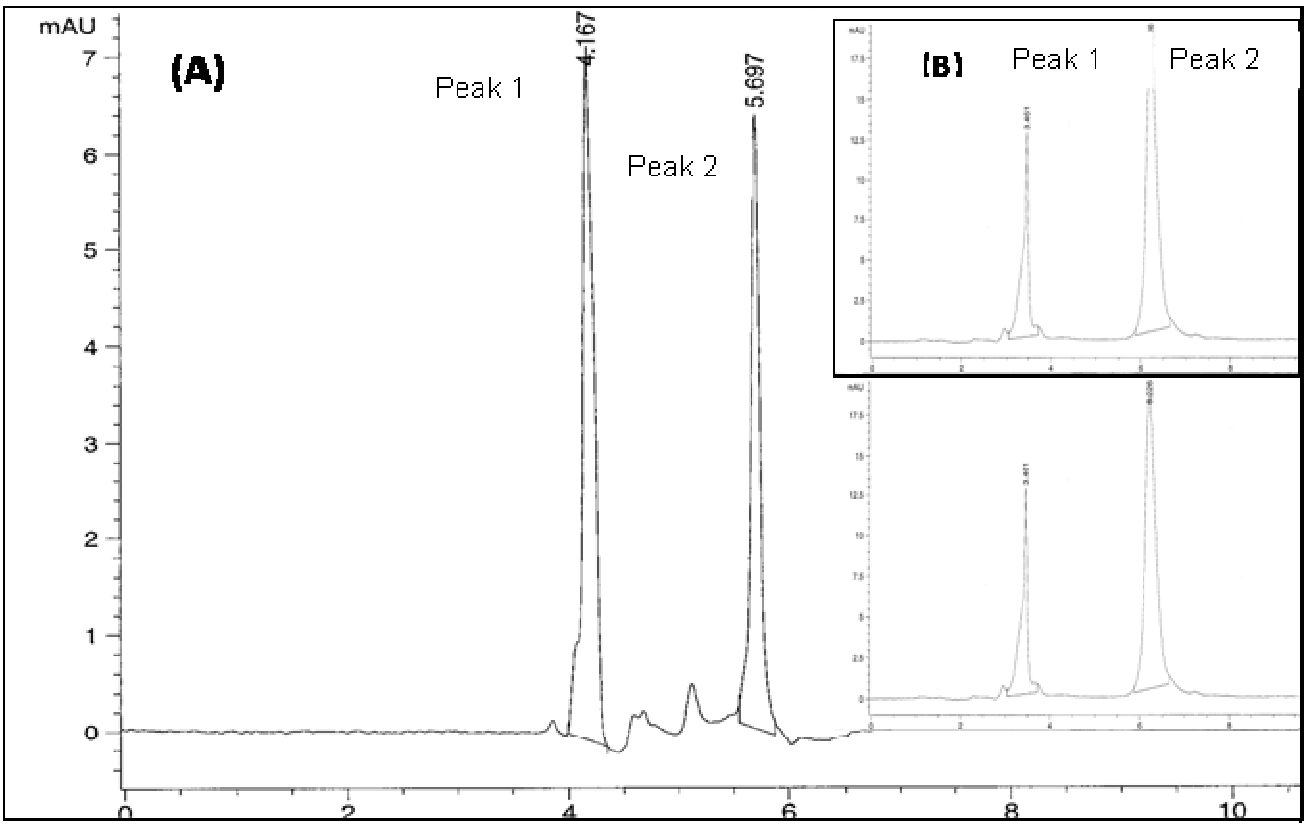

Figure 2. Separation of phytic acid using HPLC. (A), HPLC chromatogram of phytic acid extracted from rice bran; (B), HPLC chromatogram of standard phytic acid. Separation of phytic acid and inositol was carried out using C_{18} µBondapac column (250 cm × 4.6 mm) and the mobile phase was acetonitrile-water (60:40) with a flow rate of 0.5 ml/min.

et al. (1980), Camire and Clydesdale (1982) and Cilliers and Niekerk (1986) stated 2 h of extraction time with the introduction of HPLC to the analysis of phytate. Also, when the anion-exchange concept was incorporated into quantitative phytate analysis, Harland and Oberleas (1986) considered 2 h extraction period length appropriate, while Graf and Dintzis (1982) used 2 h extraction time by using 0.5 N of HCl. The different of time used in

phytic acid extraction may be due to the different extraction methods used (for example shaking, centrifugation and titration) and difference sources of phytic acid that needs different length of time for assurance of complete extraction. A linear standard curve was obtained by plotting the decrease in absorbance at 519 nm against phytate concentration as shown in Figure 1. The range for analysis was from 3 to 30 µg/ml phytate phosphorus. The value of correlation coefficient (r = -0.9867) of the calibration curve represented a strong negative relationship between absorbance and concentration of phytate phosphorus. As the value of absorbance increased, the amount of phytate phosphorus decreased. The colorimetric assay was performed according to Haug and Lantzch (1983). This method does not require any preliminary purification and may be carried out directly in crude extract. It precipitates the phytate by an acidic Fe^{3+} solution of known content and the decrease of Fe^{3+} in the supernatant was determined by its reaction with 2,2´-bipyridine to produce a light pink color. The colorimetric method was developed by Latta and Eskin (1980) based on the decoloration of the pink Fe^{3+} sulfosalicylic complex by phytate and it eliminates the need for the acid hydrolysis essential in iron-precipitation methods. The decrease in absorbance of the supernatant was due to the removal of iron from the pink complex by the sample phytate, which is proportional to the concentration of phytate presence. However, in this method, the preliminary separation of the inorganic phosphate is retained. Therefore, the extracts require purification by anion-exchange (Vucenik et al., 1998). In the conventional method, phytate is estimated either by determining the phosphorus, inositol or iron content of the precipitate, or by measuring the excess iron in the supernatant. These methods are laborious, have low sensitivity and are not specific for phytate (IP_6) due to co-precipitation of the lower phosphates of inositol. Besides, the stoichiometric ratio of phosphorus to iron in Fe (III)-IP precipitates is affected by several variables including the way in which the precipitate is washed and by the relative proportions of the individual inositol phosphates IP_1 to IP_6 present (Oberleas and Harland, 1986). The absorbance must be measured after a short defined time because the color changes with time (Vaintraub and Lapteva, 1988). Although this method provides rapid and sensitive technique for phytate determination, the limitation of this method is that phytate cannot be identified specifically because phytate and lower inositol phosphate do not have characteristic absorption spectrum (Xu et al., 1992). The phytate can be expressed only as phytate equivalent or phytate phosphorus. Phytic acid does not have a characteristic absorption spectrum in the UV or visible light region. Therefore, spectrophotometric detection of phytic acid is based on stoichiometric metal replacement reaction from coloured complex and formation of colourless iron (III)-phytate complex (Dost and Tokul, 2006).

Neutralization is a type of double replacement reaction. This reaction is complete only if the resulting solution has neither acidic nor basic properties. According to Arrhenius theory of acids and bases, neutralization is a chemical reaction in which a solution of acid is mixed with a solution of base to form salt and water. In the neutralization reaction, the acid and base properties of H^+ and OH^- are destroyed or neutralized. The H^+ atom and OH^- are combined to form HOH or H_2O (water molecules). In this study, neutralization of phytic acid extract was done to ensure that the extracting solvent is completely removed together with impurities, which causes problems such as interference with inositol phos-phate detection or rapid deterioration in column resolution as proven by Xu et al. (1992). Therefore, it will increase the method for the determination of phytic acid in the dilute extract as well as the solution after precipitation by neutralization. Ullah and Shamsuddin (1990) reported that the inhibition of large intestinal cancer is related to the pH of the solution; Na-IP_6 solution has a pH of appro-ximately 11and neutralization gave better results (Ullah and Shamsuddin, 1990). The use of an anion exchange resin to concentrate phytate and separate it from inorganic phosphate was first introduced by Harland and Oberleas (1977) using a small column. The method was accepted by the Association of Official Analytical Chemist (AOAC) in 1986. Purification of crude extracts of biological sample is usually required prior to injection onto the analytical HPLC system (Xu et al., 1992). In this experiment, phytate was purified and concentrated on anion-exchange resins (200-400 mesh, chloride form; an AG 1-X8, Bio-Rad Co., CA, USA). According to Ellis and Morris (1983), an anion exchange was identified to produce consistent analytical results while Graf and Dintzis (1982) reported that the recovery of phytic acid from the AG 1-X8 columns was 95%. The crude of sulphuric acid (H_2SO_4) extracts was diluted with water to reduce acid concentration and ensure retention of phytate on the resin. The recommended dilution of 1:25 for beans containing 1% or more phytate was used. Inorganic phosphate was subsequently eluted with 0.1 M of NaCl and the retained phytate was eluted with 0.7 M of NaCl at a flow rate of approximately 0.4 ml/min. The flow rate during elution was one of the significant factors that contributed to the recovery of phytate. Inorganic phosphate is present in seeds and the product of their processing in amounts is significantly lower than the amount of phytate (Vaintraub and Lapteva, 1988). Adjustment of the diluted aliquots to a pH of 6 before running them through the anion exchange purification is necessary to eliminate or minimize the binding effect of phytate to plant protein and minerals. Besides, it also improves the recovery of phytate, increasing the accuracy and precision of the assay. In addition, the complexes between phytate and mineral cation are pH sensitive (Fruhbeck et al., 1995). According to Ellis and Morris (1983), the recoveries of

phytate reached nearly 100% only when the pH of the extract applied to the column was adjusted to 6.0. The purification step of anion exchange considerably increased the precision and accuracy and extended the applicability of the method without affecting the results. Most impurities eluted in the neutral fractions were also discarded. The passage of the extract over a strong anion exchange seemed the best choice because of the relative ease and speed. It is also economical since the methods are extremely selective of AG 1-X8 for phytate, and it is capable to concentrate phytate from very dilute extracts (Graf and Dintzis, 1982). The recovery of phytate is comparatively independent of the volume in which phytate is applied to the column, provided the chloride ion concentration is kept at 0.1 M or below by diluting the extract (Graf and Dintzis, 1982). According to Fruhbeck et al. (1995), the use of chromato-graphic purification yielded significantly higher results than those obtained from unpurified crude extracts, hence demonstrating that reliable phytate assessment in foodstuffs demands the consumption of anion-exchange chromatography. Graf and Dintzis (1982) reported that by using AG 1-X8 anion-exchange resins, the refractive index peak due to phytate was well separated from a minor salt peak, and the area under the peak was linearity proportional to the phytate concentration over a wide range. These findings differed from the studies by Vaintraub and Lepteva (1988) who measured phytate content directly in crude extracts with and without Anion Exchange Chromatography (AEC). They also reported that by omitting the AEC, precision and sensitivity of the assay were enhanced without affecting the results. Deter-mination of phytic acid was done by reverse-phase High Performance Liquid Chromatography (HPLC). HPLC chromatograms were compared with standard phytic acid, in which two peaks were determined (Figure 2). The first peak represented phytic acid and the second peak represented inositol. Standard phytic acid was diluted into four concentrations; 50, 100, and 1000 ug/ml. The sample phytic acid was also diluted into the same concentrations. The two peaks obtained in this study were similar as obtained in the standard phytic acid. The retention time for phytic acid extracted from rice bran was 4.167 and the retention time obtained in the standard phytic acid was 3.46. Anion exchange column chromato-graphy and HPLC were shown to be best suited for the separation of inositol hexaphosphate (Xu et al., 1992). According to Harland and Oberleas (1986) the used of anion-exchange method for the determination of phytate meets the criteria of rapidity, simplicity, reproducibility, and accuracy. It is also sensitive at the lower limits of detection and otherwise compares favorably with the iron precipitation method. Studies by Sandberg and Ahderinne (1986) and Lehrfeld (1989) also proved that the anion exchange column chromatography and ion-pair HPLC methods were shown to be the best suited for the analysis of inositol phosphates in nutritional studies. These two peaks obtained were similar as shown by Tangendjaja et al. (1980) in rice bran extract. The result obtained is also in good agreement with previous reports based on the application of HPLC methodology (Sandberg and Ahderrine, 1986; Lehrfeld and Morris, 1992), and it was found that phytic acid was the major inositol phosphate in the different sample, ranging from 100% in *Lupinus albus* to 68.9% in *V. faba* cv. *Alameda.* The slight differences in the retention times between the standard phytic acid and sample phytic acid may be due to the slight differences in the ratio of mobile phase. This was in turn due to inaccuracy made during the prepara-tion of the mobile phase. According to the peak obtained, a high degree of purification on an AG 1-X8 anion-exchange resins was achieved since most impurities were eluted in the neutral fractions and discarded. The purification step also permitted the dilute extract of phytic acid to be concentrated effectively. The result obtained also increased the precision and accuracy and extended the applicability of HPLC method for the determination of phytate.

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REFERENCES

Averill HP, King CG (1986). Phytin Content of Foodstuffs. J. Am. Chem. Soc. 48: 724-728.

Boss V, Desai MA, Smith TS, Conn PJ (1992).Trans-ACPD-induced phosphoinositide hydrolysis and modulation of hippocampal pyramidal cell excitability do not undergo parallel developmental regulation. Brain Res., 594: 181-188.

Camire AL, Clydesdale FM (1982). Analysis of phytic acid in foods by HPLC. J. Food Sci., 47: 575–578.

Cilliers JJL, Van Niekerk PJ (1986). LC determination of phytic acid in food by postcolumn colorimetric detection. J. Agric. Food Chem., 34: 680-683.

De Boland AR, Garner GB, O'Dell BL (1975). Identification and properties of "phytate" in cereal grains and oilseed products. J. Agric. Food Chem., 23: 1186-1189.

Dost K, Tokul O (2006). Determination of phytic acid in wheat and wheat products by reverse phase high performance liquid chromatography. Anal. Chim. Acta, 558: 22-27.

Ellis R, Morris ER (1983). Improved ion-exchange phytate method. Cereal Chem., 60: 121-124.

Fruhbeck G, Alonso R, Marzo F, Santidrian SA (1995). Modified method for the indirect quantitative analysis of phytate in foodstuff. Anal. Biochem., 225: 206-212.

Gifford SR, Clydesdale FM (1990). Interactions among calcium, zinc and phytate with different sources. J. Food Sci., 55: 1720-1724.

Graf E, Dintzis FR (1982). High-performance liquid chromatographic method for the determination of phytate. Anal. Biochem., 119: 413-417.

Grases E, Simonet BM, Perell J, Costa-Bauz A, Prieto RM (2004). Effect of phytate on element bioavailability in the second generation

of rats. J. Trace Element in Med. Biol., 171: 229-234.
Harland BF, Oberleas D (1986). Anion-exchange method for determination of phytate in foods: collaborative study. J. Assoc. Official Anal. Chem., 69: 667-670.
Haug W, Lantzch HJ (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric., 34: 1423-1426.
Hossain M, Narciso J (2004). Global Rice Economy: Long-term perspectives. In FAO Rice Conference: proceedings: February 12-13; Rome.
Knuckles BE, Kuzmicky DD, Betschart AA (1982). HPLC analysis of phytic acid in selected foods and biological samples. J. Food Sci., 47: 1257-1262.
Latta M, Eskin M (1980). A Simple and Rapid Colorimetric Method for Phytate Determination. J. Agric. Food Chem., 28: 1315-1315.
Lee S-H, Park H-J, Cho S-Y, Jung H-J, Cho S-M, Cho Y-S, Lillehoj HS (2005). Effects of dietary phytic acid on serum and hepatic lipid levels in diabetic KK mice. Nutr. Res., 25: 869-876.
Lehrfeld J (1989). High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. Cereal Chem., 66: 510-515.
Lehrfeld J, Morris ER (1992). Over-estimation of phytic acid in foods by the AOAC anion-exchange method. J. Agric. Food Chem., 40: 2208-2210.
Liu Z, Cheng F, Zhang G (1992). Grain phytic acid in japonica rice as affected by cultivar and environment and its relation to protein content. Food Chem., 89: 49-52.
March JG, Simonet BM, Grases F (2001). Determination of phytic acid by gas chromatography-mass spectroscopy: application to biological samples. J. Chromatogr., 757: 247-255.
Oberleas D, Harland BF (1986), Anion-exchange method for determination of phytate in foods: Collaborative study. J. Assoc. Official Anal. Chem., 69: 667-670.
Oberleas D, Harland BF (2001). Newer Methods for Phytate Analysis in Handbook of Dietary Fiber in Human Nutrition, ed. by Spiller G, pp. 113-126.
Ramezanzadeh FM, Rao RM, Prinyawiwatkul W, Marshall WE, Windhauser M (2000). Effects of microwave heat, packaging, and storage temperature on fatty acids and proximate compositions in rice bran. J. Agric. Food Chem., 48: 464-467.
Ravindran V, Ravindran G, Sivalogan S (1994). Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chem., 50: 133-136.
Sandberg A-S, Ahderinne R (1986). HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. J. Food Sci., 51: 547-550.
Shamsuddin AM (2002). Anti-cancer function of phytic acid. Int. J. Food Sci. Technol., 37: 769-782.
Shamsuddin AM, Vucenik I (2005). IP_6 and Inositol in Cancer Prevention and Therapy. Curr. Cancer Ther. Rev., 1: 259-269.
Tangendjaja B, Buckle KA, Wooton M (1980). Analysis of phytic acid by high-performance liquid chromatography. J. Chromatogr., 197: 274-277.
Ullah A, Shamsuddin AM (1990). Dose-dependent inhibition of large intestinal cancer by inositol hexaphosphate in F344 rats. Carcinogenesis, 11: 2219-2222.
Vaintraub IA, Lapteva NA (1988). Colorimetric determination of phytate in unpurified extracts of seed and the products of their processing. Anal. Biochem. 17: 227-230.
Verghese M, Rao DR, Chawan CB, Walker LT, Shackeleford L (2006). Anticarcinogenic effects of phytic acid (IP_6): Apoptosis as a possible mechanism of action. LWT 39: 1093-1098.
Vucenik I, Kalebic T, Tantivejkul K, Shamsuddin AM (1998). Novel anticancer function of inositol hexaphosphate (IP6): inhibition of human rhabdomyosarcoma in vitro and in vivo. Anticancer Res., 18: 1377-1384.
Vucenik I, Shamsuddin AM (2003). Cancer Inhibition by Inositol Hexaphosphate (IP_6) and Inositol: From Laboratory to Clinic. J. Nutr., 133: 3778-3784.
Wei W, Fang-min C, Zheng-H, Ke-su W (2007). Difference of Phytic Acid Content and its Relation to Four Protein Composition Contents in Grains of Twenty-nine japonica Rice Varieties from Jiangsu and Zhejiang Provinces, China. Rice Sci., 14: 311-314.
Xu P, Price J, Agett PJ (1992). Recent Advances in Methodology for Analysis of Phytate and Inositol Phosphates in Foods. Prog. Food Nutr. Sci., 16: 245-262.
Zullaikah S, Melwita Ju Y-H (2009). Isolation of oryzanol from crude rice bran oil. Biores. Technol., 100: 299-302.