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Utilization of wheat bran as a source for phytic acid production

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Abstract

Phytic acid i.e. myo-inositol-hexakisphosphate is the most storage form of phosphorus in seeds and pollens that is a strong chelating agent of metals such as zinc, calcium, iron, magnesium, copper and potassium. This compound also has beneficial effects on human health and can act as an anti-oxidant, anti-inflammation and as energy storage. The aim of this study is to introduce a new method for phytic acid extraction from wheat bran which is a large scale by-product of milling industry with high levels of phytic acid. Following centrifugation the pH of obtained supernatant was adjusted to 8 with 1.5 M Na_2CO_3 and the obtained pellet was resuspended in 1.0 N HCl and was treated with formaldehyde and celit. Finally, following Whatman paper filtration, the pellet was dried at 60˚C for 1 day and its purity was confirmed by a spectrophotometry procedure using wade reagent. As a result the average of purity was 66.95% and average weight of obtained phytic acid was 2.94 gram per 100 gram of wheat bran. The results of IR confirmed that the obtained material is phytic acid and the characteristic peak of phytic acid was obviously present in IR analysis. The results of this research suggest that this procedure is an environmental friendly method without environmental pollution which enables us to prepare phytic acid with high yield in order to be used in food and pharmaceutical formulations.

Introduction

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6 - hexakisphosphate) is the most storage form of phosphorus in seeds and pollens. Two-thirds of the phosphorus present in cereal grains, legumes and oil seeds are in the form of phytate, but it has poor availability for monogastrics such as pig, poultry and human due to the lack of phytase enzyme for its degradation. Phytate transports through the intestine and is excreted in the feces. This causes environmental problems such as pollution by phosphorus and sudden increasing of bacteria and fungi in areas of intensive livestock farms. In addition, phytate combines with nutritionally important metals such as iron, zinc, magnesium, calcium, potassium, copper and manganese as well as with proteins and vitamins as insoluble complexes leading to decrease their utilization efficiency, activity and digestibility. In addition, some *in vitro* studies have shown that phytate-protein complexes are less attacked by protease enzymes, even some enzymes such as pepsin, amylopsin, and amylase would be repressed by phytate. Furthermore, phytate may interfere with degradation of lipids and starch (Kim *et al*., 1998; Cao *et al.*, 2007; Rao *et al.*, 2009). The daily intake of phytic acid (PA) has been estimated to be approximately 200-800 mg per individual in industrialized countries and approximately 2 gr in developing countries (Peng *et al.*, 2010). Since the 1990s, PA has been scientifically emphasized for its beneficial effects on human health, particularly in the inhibition of diabetes, renal calculi, Parkinson's disease and cancer (Canan *et al.*, 2011). Phytic acid act as an antioxidant, selective anti-inflammatory agent, energy storage, regulator of vesicles via binding to different proteins and inhibitor of pathological calcium salts crystallization (March *et al*., 2001; Peng *et al*., 2010). The undesirable crystallization processes which results in stone formation can take place in the kidney or in the urinary tracts. Experiments undertaken to display the relation between phytic acid and the crystallization of calcium salts in tissues are in their initial steps with animal experimentation. Enhancement of urinary excretion of phytic acid is offered for reduction the risk of calcium stone formation in kidney (March *et al*., 2001). It has also been reported that wheat bran, due to its endogenous and exogenous phytic acid content, when added to a low fiber diet, can reduce the early biomarkers of colon cancer (Jenab and Thompson, 2000). Studies have been showed that consumption of drinking water containing 0.5% rice bran phytic acid after tumor induction has reduced

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the risk of colon cancer in rats. Its antioxidant effects, described by several researchers, that is due to its ability to inhibit the formation of hydroxyl radicals (OH°) and to form chelates with Fe^{2+} ions, causing them to become catalytically inactive (Canan *et al*., 2011). The chelation ability of phytic acid with minerals has been suggested to have beneficial effects on reduction of serum cholesterol and triglycerids and inhibition of oxidation dependent to iron (Saad *et al*., 2011).

Wheat bran is a by-product of milling industry which is produced in large scales with high levels of phytic acid that have an antioxidant and beneficial effects on health. Since wheat is one of the most cultured crops in all parts of Iran, its bran is an excellent and available resource for phytic acid production. Therefore, this study has offered a useful technique for extraction and purification of phytic acid from wheat bran which leads to increasing nutrition value of this by-product. According to the phytic acid characteristics mentioned above, this product can be used as a food additive in order to achieve mentioned benefits.

Materials and Methods

Extraction and purification of phytic acid

Phytic acid extraction has been done according to Canan *et al*. (2011) method with to some extents modifications. At first, 20 grams of wheat bran were mixed with 200 ml of 1.0 N HCl and shaken for one hour at room temperature on stirrer. Then its pH was adjusted to 6.2 using 4.0 N NaOH (isoelectric point of wheat proteins that maximum of wheat bran proteins will precipitate in this pH. Wrigley and Bietz reported that gluten proteins are insoluble near their isoelectric point) and centrifuged in 3000 rpm for 10 min (Wu, 1993). Then the pH of supernatant was adjusted to 8 with 1.5 M Na_2CO_3 solution and remained for 12 hours at room temperature. After centrifugation at 3000 rpm for 10 min, the supernatant was decanted and the obtained pellet was resuspended in 1.0 N HCl. In order to remove contaminants and protein denaturation, 10 ml formaldehyde and 0.5 gram celit was added. This suspension was then shaken for 2 hours and remained for 12 hours at room temperature. The prepared suspension was then passed through qualitative Whatman filter paper No. 3 and the pH of filtrate was adjusted to 7 by 1.5 M Na_2CO_3. Finally, the formed pellet was recovered through filtering by qualitative Whatman filter paper No. 3 and dried at 60˚C for 1 day (Canan *et al*., 2011). Canan *et al.* (2011) utilized rice bran to extract phytic acid which its protein isoelectric point is 4.5 that it is the difference of their study with present study.

Determination the purity of phytic acid

There are several methods for detection of phytic acid concentration in cereal products, biological and urine samples that in this study a cost effective procedure i.e. spectrophotometry was used. Standard solutions containing 5-40 µg/ml phytic acid (Sigma, USA) in distilled water were prepared. Three milliliters of the standard solutions were poured into experimental tubes and a tube containing 3 ml of water also used as blank. To each tube 1 ml of the modified wade reagent (0.03% $FeCl_3.6H_2O$ and 0.3% sulfosalicylic acid in distilled water) was added and the solution was mixed through vortexing for 5 seconds. The mixture then was centrifuged for 10 min and the absorbance of supernatant was read at 500 nm. Finally, standard curve was drawn by Microsoft Excel software. All of the procedures were also repeated for obtained phytic acid and its concentration determined according to standard curve. This experiment was repeated 3 times (Latta and Eskin, 1998).

Determination of phytic acid by IR

The quality of obtained phytic acid was determined by FTIR (Fourier transform infrared spectroscopy) using BOMEM MB102 apparatus in 400 cm^{-1}-4000 cm^{-1} range.

Results

The extracted phytic acid has white crystal appearance which its standard curve was shown in Figure 1. As it can be found, the standard curve has descending pattern. The reason is decreasing the pink color intensity following to the addition of reagent to phytic acid because in the presence of phytate iron bounds to the phosphate ester and so is unavailable to react with sulfosalicylic acid (Latta and Eskin, 1998). The Purity and yield of purified phytic acid was measured in 3 repeats. The mean results of these 3 repeats showed that this extraction method can provide 2.94 gr phytic acid per 100 gr of wheat bran with 66.95 % purity. An analysis result of extracted phytic acid was shown in figure 2.The results of IR confirm that the extracted material is phytic acid.

The results showed that the average purity of extracted phytic acid was 66.95% and the average yield of extracted phytic acid was 2.94 gram per 100 gram of wheat bran. In this method the availability of obtained phytic acid is .0294 gram per 1 gram wheat bran. This method introduces a low cost method with minimum requirements that can be used

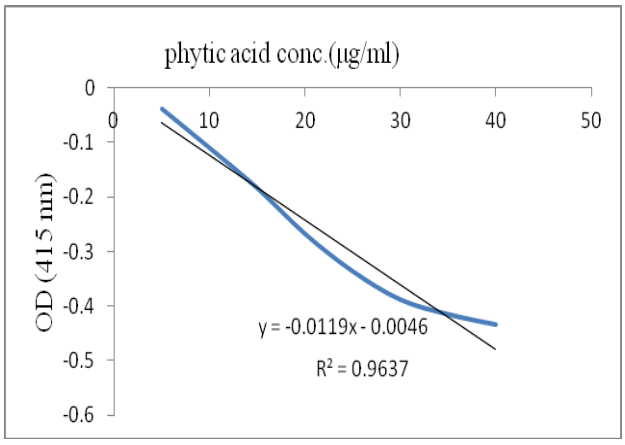

Figure 1. Standard curve of phytic acid

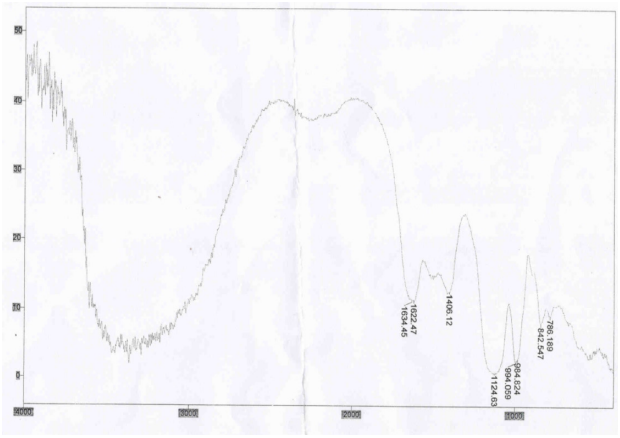

Figure 2. IR of extracted phytic acid

for extraction of phytic acid by acceptable purity from wheat bran as agricultural waste. So, through processing this product it is possible to obtain a significant source of phytic acid that can be used in food and pharmaceutical formulations.

Discussion

Phytic acid is one of the active compounds that have been studied in order to discover their effects on health (Saad *et al*., 2011). In this study, hydrochloric acid was used for phytic acid extraction from wheat bran. According to Gifford and Clydesdale (1990) results, the low pH value between 0 and 1 is needed to separate phytate from iron and protein complexes (Gifford and Clydesdale, 1990). Saad *et al*. (2011), in their study utilized three acidic solutions including trichloroacetic acid, hydrochloric acid and sulfuric acid for extraction of phytic acid from rice bran. Their results showed that 5% H_2SO_4 after 30 min of the beginning of extraction resulted the highest yield of phytic acid comparing to 10% TCA and 3% HCl. Phytic acid content in rice bran has been optimized from 0.22 to 2.22% for different parameters (Saad *et al*., 2011). Kumar *et al.* (2011), extracted the phytate from defatted Jatropha kernel meal using organic solvents (acetone and carbon tetrachloride) with 66% phytate and 22% crude protein (Kumar et al., 2011). Canan *et al.* (2011), purified phytic acid from rice bran that according to their reports, HCl concentration, temperature and time had significant effects on the extraction procedure that in such way 1.0 N HCl, 25˚C temperature and 1 h were the best conditions for phytic acid extraction. Canan *et al.* (2011) obtained 3.85 gram phytic acid per 100 gram rice bran which is more than the yield of phytic acid obtained in this study that is due to differences in the type of bran and extraction conditions (Canan *et al*., 2011).

Extracted phytic acid in the present study, has poor solubility in distilled water and neutral pH that is consistent with Canan *et al.* (2011) results. They reported that the solubility of purified phytic acid was reached at pH 4.0, 6.0 and 8.0, but solubility of phytic acid in pH 4.0 was higher than others. The low solubility value of the purified phytic acid at pH 6.0 to 8.0 was possibly due to the presence of other salts, particularly calcium phytates and carbonates associated with zinc and copper phytates, which have a lower solubility at a pH near 6.0 (Canan *et al*., 2011). According to Graf *et al*. (1987), phytic acid solubility in different values of pH is important for its application as an antioxidant in foods because phytic acid is an effective and nontoxic iron chelating agent (Graf *et al*., 1987).

There was a possibility that formaldehyde residue have present in final phytate, however considering this fact that its boiling point is -19˚C, therefore it was volatilized and also the conditions of drying to obtained phytate at 66˚C for 24 h should be enough to remove this residue (Canan *et al*., 2011). Since the solubility of obtained phytic acid is very poor in neutral pH, thus for elimination of formaldehyde and other unlinked salts to phytic acid, the obtained pellet can be washed by a little amount of distilled water, therefore the obtained pellet will be whiter with higher quality.

Conclusion

The results of this research showed that this procedure can extract the high-cost phytic acid from wheat bran by low facilities and simple procedures such as centrifugation, filtration and pH adjustment which this material can be used in food and pharmaceutical formulations. Furthermore, the used procedure is environmental friendly and doesn't cause environmental pollution which is a high advantage for large scale production of this compound.

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