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Phytic Acid

Key Words: Phytic acid; myoinositol; Wade reagent; metal ions chelater; Amberlite AG1-X8 anion exchange resin; antioxidant; legumes; cereals; phytate precipitation.

1. Introduction

1.1. Nature, Mechanism of Action, and Biological Effects

Phytic acid, a cyclic compound (1,2,3,4,5,6-hexakis dihydrogen phosphate myoinositol) is a common storage form of phosphorus in seeds and is also considered as an antinutritional factor. Phytic acid, as a result of possessing negative charge at a wide range of pH values, has strong affinity to bind metal ions such as with calcium, zinc, and iron (**Fig. 1**). This leads to interference in the absorption of these minerals from small intestine and adversely affects various metabolic processes. In addition, phytic acid is also known to complex with proteins and starch, resulting in reduced digestibility of these nutrients. The phosphorus in phytic acid is not nutritionally available to monogastric animals. Nonetheless, non-antinutritive concentration of phytic acid in dietary sources is recently considered to be a potential antioxidant. Reduction in iron-induced oxidative injury and reversal in initiation of colorectal tumorigenesis have also been observed. Phytic acid has recently been suggested to have a protective role in carcinogenesis.

1.2. Present in

Glycine max, Cicer arietinum, Vigna mungo, Vigna radiata, Entada scandens, Cajanus cajan, Lablab purpureus, Lens culinaris, Phaseolus lunatus, Phaseolus vulgaris, Moringa oleifera, Jatropha curcas, Entada scandens, Sesbania sesban, Sesbania bispinosa, Triticum vulgare, Mucuna pruriens, Vicia

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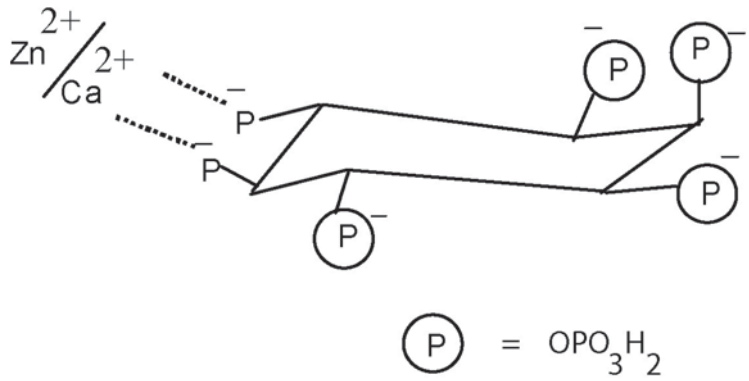

Fig. 1. Interaction of phytic acid, hexadihydrogenphosphate with metal ions.

faba, Vigna aconitifolia, Treculia africana (African breadfruit), *Artocarpus altilis* (Polynesian breadfruit), and rapeseed.

1.3. Principle of Assays

This chapter presents two methods for determination of phytic acid (phytate).

In the first method, phytate is extracted with trichloroacetic acid and precipitated as ferric salt. The iron content of the precipitate is determined spectrophotometrically and the phytate phosphorus content is calculated from this value, assuming a constant 4 Fe/6 P molecular ratio in the precipitate ***(1)***.

In the second method, phytate is extracted with 3.5% [weight/volume (w/v)] HCl and further purified through an AG1–X8 chloride anion exchange column. The pink color of the Wade reagent is due to the reaction between ferric ion and sulfosalicylic acid with an absorbance maximum at 500 nm. In the presence of phytate, the iron becomes bound to the phosphate ester and is unavailable to react with sulfosalicylic acid, resulting in a decrease in pink color intensity ***(2)***.

2. Materials

2.1. Method 1: Based on Precipitation of Phytate (1)

1. *Trichloroacetic acid (TCA), 3%*. Weigh 3 g TCA and dissolve in 100 mL distilled water.
2. *Sodium sulfate (3%) in 3% TCA*. Weigh 3 g sodium sulfate and dissolve in 100 mL of 3% TCA.
3. *NaOH (1.5M)*. Weigh 6 g sodium hydroxide and dissolve in 100 mL distilled water.
4. *HNO_3 (3.2N)*. Take 20.5 mL nitric acid and make the volume up to 100 mL with distilled water.

5. *$FeCl_3$ solution.* Dissolve 583 mg $FeCl_3$ in 100 mL of 3% TCA.
6. *Potassium thiocyanate (KSCN), 1.5M.* Dissolve 29.15 g of potassium thiocyanate in 200 mL distilled water.
7. *Stock standard $Fe(NO_3)_3$ solution.* Weigh 433 mg $Fe(NO_3)_3$ and dissolve in 100 mL of distilled water in a volumetric flask.

2.2. Method 2: Based on Reaction with Wade's Reagent (2)

1. *HCl (3.5% w/v).* Take 50 mL of concentrated HCl (37% w/v) and dilute to 529 mL with distilled water.
2. *NaCl (0.7M).* Dissolve 40.91 g NaCl in 1 L of distilled water.
3. *NaCl (0.1M).* Take 100 mL of 0.7 M NaCl solution and add to it 600 mL of distilled water.
4. *Wade reagent.* Take 30 mg of $FeCl_3.6H_2O$ and 300 mg of sulfosalicylic acid in a 100-mL volumetric flask, dissolve in approximately 70 mL distilled water, and make the volume up to 100 mL with distilled water.
5. *Amberlite AG1–X8 (200–400 mesh) anion-exchange resin.* Take 0.5 g of resin (commercially available; Bio-Rad Laboratories, Richmond, CA) and fill in a 0.75 cm × 25 cm column plugged with a small quantity of glass wool.

3. Methods

3.1. Method 1

1. Weigh a finely ground (40 mesh, ground preferably using a ball mill) sample estimated to contain 5 to 30 mg phytate-P into a 125-mL Erlenmeyer flask. Generally, the amount weighed for cereals and legumes is 500 to 700 mg.
2. Extract phytate in 50 mL of 3% TCA by shaking on a magnetic stirrer for 30 min or with occasional swirling by hand for 45 min.
3. Centrifuge the suspension (3000 *g*, 10 min) and transfer a 10-mL aliquot of the supernatant to a 40-mL conical centrifuge tube.
4. Add rapidly 4 mL of $FeCl_3$ solution to the aliquot in the centrifuge tubes. Heat the contents in a boiling water bath for 45 min. If the supernatant is not clear after 30 min, add one or two drops of 3% sodium sulfate in 3% TCA and continue heating.
5. Centrifuge (3000 *g*, 10–15 min) and carefully decant the clear supernatant. Wash the precipitate twice by dispersing it well in 20 to 25 mL 3% TCA. Heat it in boiling water for 5 to 10 min and then centrifuge (3000 *g*, 10 min). Repeat the washing of the precipitate with distilled water.
6. Disperse the precipitate in a few milliliters of water and add 3 mL of 1.5N NaOH with mixing. Bring volume to approximately 30 mL with distilled water and heat in boiling water for 30 min.
7. Filter hot (quantitatively) through a moderately retentive paper (Whatman No. 2). Wash the precipitate with 60 to 70 mL of hot distilled water and discard the filtrate.

8. Transfer and dissolve the precipitate that is on the filter paper into the 100 mL volumetric flask containing 40 mL of hot 3.2N HNO_3. Wash paper with several portions of distilled water and collect the washings in the same flask.
9. Cool flask and contents to room temperature and bring the volume to 100 mL with distilled water.
10. Transfer a 5-mL aliquot to another 100-mL volumetric flask and dilute to approximately 70 mL with distilled water.
11. Add 20 mL of 1.5M KSCN and bring the volume to 100 mL with distilled water, and read the color immediately (within 1 min) at 480 nm using a spectrophotometer.
12. Run a reagent blank with each set of samples.

3.1.1. Preparation of $Fe(NO_3)_3$ Calibration Curve

Take 2.5 mL of the stock $Fe(NO_3)_3$ solution and make the volume up to 250 mL in a volumetric flask. Pipette 2.5-, 5-, 10-, 15- and 20-mL aliquots of this working standard into a series of 100-mL volumetric flasks and dilute them to approximately 70 mL with distilled water. Then proceed from step 11 in **Section 3.1**.

3.1.2. Calculation

Determine the micrograms of iron present in the test from the calibration curve, and calculate the phytate P as per the following equation:

$$\text{Phytate P mg/100 g sample} = [\text{Fe } (\mu g) \times 15]/\text{Weight of sample in g}$$

Correct the values obtained for dry matter of the sample.

3.2. Method 2

1. Extract 5 g of plant materials (40 mesh, ground preferably using a ball mill) with 100 mL of 3.5% HCl for 1 h at room temperature using a magnetic stirrer. Centrifuge the contents at 3000 *g* for 10 min at room temperature and collect the supernatant.
2. Dilute an aliquot, between 1 mL and 5 mL of the supernatant (depending on the level of phytate) to 25 mL with distilled water (*see* **Note 1**). Pass 10 mL of the diluted sample extract through an AG1 X8 chloride anion exchange (200–400 mesh) column (0.5 g) (*see* **Note 2**).
3. Inorganic phosphorus and other interfering compounds are eluted with 15 mL of 0.1M NaCl, and subsequently the phytate is eluted with 15 mL of 0.7M NaCl ***(3)***.
4. Take 3 mL of the above-eluted sample in a separate test tube and add 1 mL of the Wade reagent. Vortex and then centrifuge the mixture at 3000 *g* for 10 min. Measure the absorbance value at 500 nm against a reagent blank.

3.2.1. Preparation of Calibration Curve

Sodium phytate (for example, from Sigma, St. Louis, MO) is used as a standard since it is soluble in water and does not require conversion to free phytic acid. Prepare a series of standard solutions containing 5 to 40 µg/mL phytic acid in distilled water (*see* **Note 3**). Pipette 3 mL of the standard solution into 15-mL conical centrifuge tubes. The blank tube contains 3 mL of distilled water. To each tube add 1 mL of the Wade reagent. Mix the solution on a vortex mixture for 5 s. The mixture is centrifuged at 3000 *g* for 10 min and the absorbance of the supernatant is read at 500 nm by using water to zero the spectrophotometer ***(2)***.

4. Notes

1. If samples contain less than 1% phytic acid, a dilution of 5 : 25 in distilled water is recommended, whereas a 1 : 25 dilution is enough for samples containing 1% or more phytic acid.
2. If the interference substances are negligible either in the parent extracts or in the diluted extracts, the purification of phytic acid through a 200- to 400-mesh AG1 X8 anion exchange column is not necessary and the direct assay of phytic acid can be conducted.
3. One hundred grams of sodium phytate equals 59.9 g of phytic acid.

References

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