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MODIFICATION OF RAPID METHOD FOR PHYTIC ACID DETERMINATION IN WHEAT AND ITS PRODUCTION

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Phytic acid, present in cereals and grain legumes, is determined by several procedures involving different principles. The most common and practical is the one with extraction of phytic acid with acid solution, reaction with iron solution of known concentration and back determination of the free iron. Back determination is carried out using the 2,2-bipyridine reagent. This, besides being expensive, has several other demerits. The determination is time bound, measurement is possible in only a limited range of concentrations and the concentration dependency of the procedure is relatively low. The procedure was modified by replacing the 2,2-bipyridine procedure with potassium thiocyanate after making the necessary procedural adjustments. The curve with the pyridine procedure becomes parallel to X-axis at concentrations beyond 30µg/ml of P-Phytate; whereas that of the KSCN procedure remains straight line, indicating its applicability in even higher concentration ranges. The procedure was validated using recovery studies.

Key words: Phytic acid, Cereals, Method modification.

Introduction

Phytic acid is myo-inositol-1, 2, 3, 4, 5, 6-hexakis-dihydrogen phosphate. In most seeds it serves as primary phosphorous and myo-inositol reserve. It also stores other cations and energy (Cosgrove 1966). Binding of phytic acid with minerals is pH dependent; and complexes with different cations have varying solublities. Phytic acid also binds protein molecules (Shwenke *et al* 1986; Mothes *et al* 1987) leading to decreased solubility, functionality and digestibility of proteins (Maga 1982).

Binding of minerals with phytic acid also results in their reduced physiological availability (Cosgrove 1966). It is known to decrease the bioavailability of Ca, Fe, P, Zn and other trace elements to humans and mono-gastric animals. This leads to severe nutritional and consequently health problems in the consuming populations (Smith and Circle 1978; Maga 1982; Thompson 1987). Iron deficiency anemia (IDA) is a severe problem of public health significance in Pakistan. Quite a large proportion of various population groups are affected (Anon 1988), resulting in low Hb levels, reduced working and mental capabilities and ultimately in great economic losses (Joseph 2000). Phytic acid is considered to be one of the most significant confounder in this connection. Logically any R & D endeavor to improve the micronutrient nutriture of the population will include a component on phytic acid research.

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Several methods have been reported for the determination of phytic acid. However, a rapid and sensitive method, suitable for screening large numbers of samples, is still required. In the conventional method (McCance and Widowson 1935) the phytic acid is precipitated as ferric phytate. The precipitate is separated, washed, and the phosphorous content determined after hydrolysis. The phytic acid content is calculated from the phosphorus content. This method is very time consuming.

A relatively faster method was developed by Haug and Lantzsch (1983), by modifying an indirect method originally described by Young (1936). The sample extract is heated with an acidic Iron III solution of known iron content. The decrease in iron (determined colorimetrically with 2,2-bipyridine) in the supernatant is a measure for the phytic acid content. This method has several shortcomings at the practical level and we had to face problems of reproducibility of results while using that procedure. Color developed with pyridine reagent fades down quickly and hence the time of taking absorbance (OD) influences the results. Line drawn to plot the concentration against OD becomes parallel to X-axis beyond 30 ppm concentration, indicating in sensitivity of the procedure in that range. Apart from using expensive reagent (2,2-bipyridine), the method is not recommendable for large number of samples. A need therefore was felt for developing a method with high degree of reproducibility and sensitivity in larger concentration ranges. Since iron can be determined with

potassium thiocyanate in acidic medium (Ranganna 1978), an attempt was made to replace the 2,2-bipyridine procedure by determining the excess iron in solution, using thiocyanate as chromogen. Present paper reports results of different tests performed in these studies.

Materials and Methods

Materials. The following solutions are necessary:

(I) Phytate reference solution. Stock solution was prepared which contained 0.15g sodium phytate (Sigma Can. No P-5756) in 100 ml de-ionized water. The reference solutions were prepared by diluting the stock solution with 0.2N HCl in a range from 3-30 µg/ml phytate phosphorus.

(II) Ferric solution. 0.2g ammonium iron (iii) sulfate $12H_2O$ (Merck Art. 3776) was dissolved in 100 ml 2N HCl and volume made up to 1000 ml with de-ionized water.

(III) 15% w/v Potassium thiocyanate (KSCN) solution. 15g of KSCN was dissolved in sufficient de-ionized water and make the volume up to 100 ml.

(IV) 2,2-Bipyridine solution. 10g of 2,2-bipyridine (Merck Art 3098) was dissolved in de-ionized water and 10 ml thioglycollic acid (Merck Art 700) was added and the volume was made up to 1000 ml.

Procedure. Wheat sample (≅ 0.06g) was extracted with 10 ml of 0.2N HCl. An aliquot of 0.5 ml of this extract was taken into a 10 ml test tube, fitted with a ground glass stopper. Solution-II (1 ml) was added to it. To compare the two procedures, phytic acid was determined in the extract by both the KSCN-as well as 2,2-bipyridine procedure.

KSCN-procedure. De-ionized water (2 ml) was added to the test tube. The tube was covered with stopper and fixed with a clip. The tubes were heated in a boiling water bath for 30 min. Care was observed for the first 5 min that the tubes remain well stoppered. After cooling in ice water for 15 min the tubes were allowed to adjust to room temperature. Solution-III (0.25 ml) was added and the contents mixed. Absorbance (OD) was taken at 480 nm after 25-30 min.

Bipyridine procedure. No de-ionized water was added. The tube was covered with stopper, fixed with a clip. The tube was heated in a boiling water bath for 30 min. Again care was taken for the first 5 min that the tubes remain well stoppered. After cooling in ice water for 15 min it was allowed to adjust to room temperature. Solution IV (2ml) was added to the test tube. The OD at 519 nm must be taken immediately (0.5-1.0 min) because the bipyridine reacts with iron phytate and therefore the color fades with time.

Results and Discussion

Standard series of Na-Phytate solution was prepared and calibration curves were developed with both procedures (Fig 1-2). The curve developed with bipyridine procedure was linear in the concentration range of 3-30 µg/ml phytate phosphorus. With higher concentrations, the line became parallel to X-axis. The curve reported by Haug and Lantzsch (1983) also exhibited a similar behavior in the concentration range above 30 µg/ml. This indicated in sensitivity and hence non applicability of the procedure in that range of phytate concentrations. On the other hand the standard curve developed with the KSCN procedure, remained linear up to much higher concentrations, showing that this method is more sensitive to a wider range of concentrations than the 2,2-bipyridine procedure. As with the pyridine procedure, the modified method also relies on back determination of surplus added iron in the system. The ferric ions form the red brown ferrithiocyanate complex with KSCN in acidic conditions (Ranganna 1978) and is measured spectrophotometrically at 480nm. This complex is stable in acidic medium and therefore does not fade with time, leading to freedom from time dependency.

Recovery studies (Table 1) were conducted with the KSCN procedure. The method was tested with extracts of wheat.

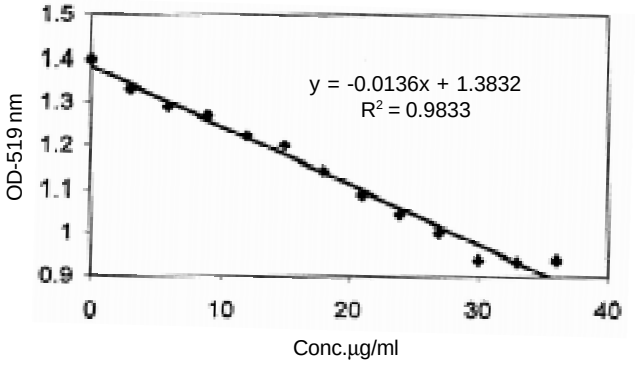

Fig 1. Standard curve for P-phytate 2-2, Bipyridine method.

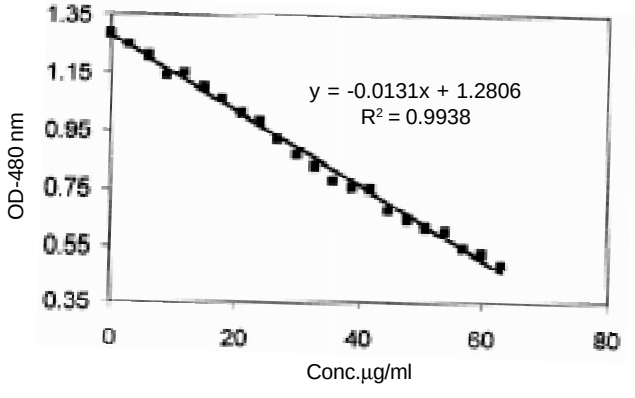

Fig 2. Standard curve for P-phytate KSCN method.

Table 1
Recovery of added P-phytate using KSCN procedure

Added P-phytate (ppm)	P-phytate in solution (ppm)	P-phytate recovered (ppm)	Recovery %
0	13.8	–	–
20	33.7	19.9	99.5
30	43.6	29.8	99.2
40	53.05	39.7	99.2
45	58.4	44.6	99.2
		Mean recovery	99.3

Wheat samples grinded to pass through a 30-mesh screen were extracted (0.06g in 10 ml of 0.2N HCl). Graded amounts (20, 30, 40, 45 µg/ml) of phytate phosphorous were added to the extracts in triplicate and determined back. The average recovery of added P-phytate in all the concentrations was 99.3%.

The modified (thiocyanate) procedure has advantages over the already existing methods. The method of McCance (1935) was time consuming, with lot of washing work of the precipitate. That makes it impractical to analyze many samples. It therefore cannot be recommended for routine analysis, involving large number of samples. The method of Haug and Lantzsch (1983), though rapid, depends on the color developed by the reaction of iron with 2,2-bipyridine. It has two disadvantages working in opposite directions. The reaction is not limited to the free iron in the solution, and with passage of time the pyridine reagent may react with the phytate iron. This factor may result in increased color intesity, making the phytate determination ineffective. Acting in the opposite direction is the fact that the color of iron-pyridine complex fades down with time, resulting in yet another uncertainty about the results.

In contrast to this, the thiocyanate procedure has the advantages of color stability in acid medium, and does not react with the phytate bound iron, and hence yields reliable results. Also the reagent yields measurable color with very low concentrations of iron. Hence in samples with relatively high phytate concentrations, very little iron is left free in the solution. Thus the KSCN procedure works reliably in this range of concentrations, whereas the bipyridine reagent fails to develop color in low concentration of iron. The modified procedure will be of great interest and use for those engaged with research in cereals and specifically those working with micronutrient.

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