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Rapid and sensitive liquid chromatographic method using a conductivity detector for the determination of phytic acid in food

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Abstract

An LC method was developed for the determination of phytic acid in food. The separation was carried out by gradient elution on an anion-exchange column using a conductivity detector. Earlier reversed-phase LC procedures for the quantitation of phytic acid usually required a prepurification step. The prepurification can be avoided by the separation method described in this paper. The method is sensitive and selective, and can be rapidly and easily performed. It is therefore suitable for routine determination. © 1998 Elsevier Science B.V.

Keywords: Food analysis; Phytic acid

1. Introduction

Most plants contain phytic acid salts (phytic acid or [myo-inositol 1,2,3,4,5,6 hexakis(dihydro phosphate)] acid) (Fig. 1). These salts are mainly present in grains and seeds of which they represent 1 to 5% of the dry matter content, and account for 50–90% of total phosphorus [1]. Particularly abundant in leguminous plants and cereals, phytates contribute to the reduction of the bioavailability of divalent minerals such as Ca, Mg, Fe, Zn, Cu, Co [2]. Moreover, phytates also interact with proteins by making stable bonds [3]. They can hence be considered as potential enzyme inhibitors [4]. The degradation processes of phytates have been well studied: they usually involve chemical and enzymic dephosphorylation reactions during germination [5] and fermentation [6]. The degradation of phytic acid leads to mono- to pentakisphosphates whose anti-nutritional effects increase

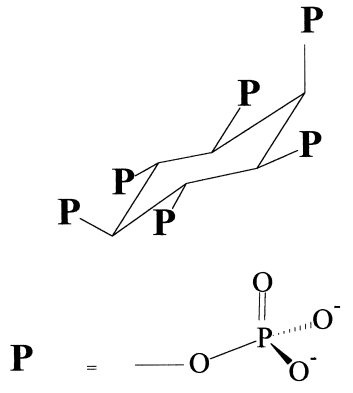

Fig. 1. Structure of inositol 1,2,3,4,5,6-hexaphosphate (or phytic acid).

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with the phosphorylation degree [7]. A rapid and reliable method for the quantitation of phytic acid and its salts in food is therefore required for the evaluation and improvement of food nutritional value. These aspects are particularly important for foods that constitute the staple of groups of population deficient in essential minerals, such as infants and young children in developing countries [4].

Three types of analytical method are available for the quantitation of phytic acid: gravimetric methods, volumetric methods [8,9] and methods involving column liquid chromatography (LC).

Early applications of reserved-phase LC [10,11] used either refractometric or spectrophotometric detection. Refractive index detection was further improved by sample prepurification [12,13]. Rounds and Nielsen [14] described another method involving separation on an anion-exchange column followed by derivatization with a chromophoric reagent ($FeCl_3$-sulfosalicylic solution). Detection was carried out at 500 nm. These additional steps (prepurification and derivatization) are time consuming. As phytates are highly ionized, our separation was performed with an anion-exchange column and detection was ensured with a conductivity detector. This system of detection was shown by Scott [15] to be highly sensitive.

The purpose of this paper was to develop a more rapid procedure for phytic acid assay based on separation with anion-exchange column with a conductivity detector. The main performance features of this method were examined and tests were performed to ensure the validity of the analytical results.

2. Experimental

2.1. Materials

2.1.1. Reagents

Reagents were of analytical and chromatographic grade: methanol (Carlo Erba, Nanterre, France, Ref. 525102); isopropanol (Carlo Erba, Ref. 415154); sulphuric acid (E. Merck, Darmstadt, Germany, Ref. 100731); aqueous sodium hydroxide 50% solution (Baker, Deventer, Netherlands, Ref. 7067) and sodium phytate (Sigma, St. Louis, MO, USA, Ref. 3168) were used as standard. Water was deionized (18 MΩ) by a Milli-Q system (Millipore, Bedford, MA, USA).

2.1.2. Instrumentation

LC analyses were performed with a 4500i Dionex (Sunnyvale, CA, USA) liquid chromatograph equipped with an eluent delivery pump, an autoinjector and a conductivity detector. A 200-μl constant volume injection loop was used throughout.

Data collection and handling were carried out by a Dionex AI450 software.

A centrifugal evaporator RC10.10 (Jouan, Saint-Nazaire, France) fitted with a refrigerated trap cooled at −60°C (Jouan RCT60) was used for concentration of extract. Sample solutions were diluted with a Gilson (Middleton, WI, USA) semi-automatic dilutor (401 Dilutor) prior to injection.

2.1.3. Sample preparation

Flours were prepared with germinated and ungerminated seeds of cowpea (*Vigna unguiculata* spp.) and millet (*Pennisetum* spp.) varieties cultivated in Senegal. The seeds were first soaked in water for 24 h and ungerminated at 30°C for 48–72 h. They were then dried at 45°C for about 48 h, finely ground and passed through a 0.5-mm sieve. These samples were used to study the specificity, linearity range, accuracy, precision and sensitivity of the analytical method described [16,17].

2.2. Methods

2.2.1. Phytate extraction

0.2 g of flour was introduced in a pyrex vial with a PTFE screw-cap. Ten ml of 0.5 *M* HCl were added and the vial was capped. The mixture was heated under stirring for 5 min by immersing the vial in boiling water and then centrifuged at 4000 *g* for 10 min. The supernatant was recovered and 1.5 ml of 12 *M* HCl was added to obtain a 2 *M* HCl concentration in order to ensure the decomplexation of phytates. The resulting solution was then shaken and evaporated to dryness with a centrifugal evaporator. The vial was finally stored at 8°C.

Ten min before chromatography, the residue was diluted in 1 ml of deionized water and filtered through a 0.2-μm disposable filter (Acrodisc) tip-syringe assembly. The filtrate was then diluted in

Table 1
Gradient elution program for the separation of phytic acid

Elution time (min)	Flow-rate (ml/min)	%A	%B	%C
0.0	1	35	2	63
2.0	1	54	2	44
9.5	1	55	2	43
10.5	1	35	2	63
15	1	35	2	63

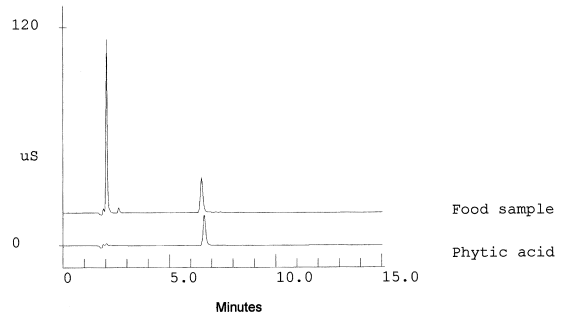

Fig. 2. Elution profile of phytic acid standard[a] and of the food sample[a]. [a] Separation of phytic acid on a Omnipac Pax-100 column; eluents: 200 m*M* NaOH, water–isopropanol (1:1, v/v) and water (18 mΩ); detection: chemically suppressed conductivity using an ASRS-I 4 mm.

deionized water (1:50) and injected into the liquid chromatograph.

2.2.2. Chromatographic conditions

The separation was carried out with an Omnipac Pax-100 anion-exchange column (25 cm×4 mm I.D.; Dionex) equipped with an Omnipac Pax-100 (8 μm) pre-column and an anion suppressor (ASRS-I 4 mm). Each eluent of the mobile phase was previously degassed in an ultrasonic bath and then introduced in the eluent delivery system under helium pressure.

The separation was performed by gradient elution using three solvents: solvent A=200 m*M* NaOH solution; solvent B=deionized water–isopropanol (1:1, v/v); solvent C=deionized water.

After several attempts, the gradient elution programme shown in Table 1 was selected with a total run time of 15 min. The anion suppressor was continually regenerated with a 50 m*M* sulphuric acid solution. Several solutions of phytic acid with a concentration from 0.01 to 0.16 m*M* were prepared from a standard solution by external calibration.

3. Results and discussion

The present approach for phytic acid analysis based on separation with an anion-exchange column and conductivity detection using three solvents for gradient elution and the performance criteria of the method are discussed below.

The plot of phytate concentration versus peak area in the range 0–0.16 m*M* was linear [R^2>0.9992 with a slope (n=4) a=13983±98]. The retention time of phytate was 6.0±0.2 min with no day-to-day variation over a 5-month period, as shown in Fig. 2.

The experimental bias was determined by analysing a reference cowpea sample extract to which a standard solution of phytic acid was added. Three identical solutions (5 ml) were prepared from the reference sample and 1 m*M* standard solution of phytic acid (120 μl). The analyte recovery ranged between 109% and 99%.

To determine recovery 15 replicate extractions were performed using the standard solution of phytic acid (3.25 m*M*). The mean concentration value recovered was 3.22±0.11 m*M*, representing 99% with a (95%) confidence range of 3.15–3.28 m*M*.

After calculating the calibration plot and prognosis interval, the limits of detection and quantification were evaluated graphically according to Miller and Miller [19]. The limit of quantification was determined by analysing phytic acid solutions of five different concentrations (0.02 m*M*, 0.01 m*M*, 0.005 m*M*, 0.002 m*M*, 0.1 μ*M*). The results of the regression analysis was: Y=12354X with R^2=0.9991. The high-performance liquid chromatography (HPLC) method therefore allows the quantitation of phytic acid down to 0.1 μ*M*. The signal-to-noise ratio was higher than 10: the limit of detection was therefore less than 0.0001 μ*M*.

3.1. Application

Methods previously published usually involved samples of 0.2 g dissolved in 10 ml of 0.5 *M* HCl solution [14–18]. The exchange capacity of the column used for prepurification not being a limiting

factor, the maximal quantifiable amount was therefore determined. Ungerminated cowpea samples were prepared in various concentrations (0.05–0.30 g in 10 ml of 0.5 *M* HCl). The measured concentrations of phytic acid (*Y*, m*M*) were plotted against the amounts added to samples (*X*, g). The graph (Fig. 3) showed a good linear relationship up to 0.2 g of phytic acid. The results of the regression analysis performed on the five points: $Y=8.9153X$ with $R^2=0.9966$. The optimum phytic acid concentration in cowpea is 2 m*M*, corresponding to about is 0.2 g of sample. Volume of solvent was only 10 ml, as compared to 30 ml in reversed-phase technique, thus permitting concentration to 1 ml final volume within a shorter time.

To determine the method reproducibility, six different samples from the same batch of ungerminated cowpea (flour) were repeatedly analyzed daily for four days. Precision for replicate injection ($n=6$) was 5% [relative standard deviation (R.S.D.) of repeatability] and 7% (R.S.D. of reproducibility).

Four samples of germinated and ungerminated cowpea and millet were analyzed. The sensitivity was determined by calculating the confidence limits [$\mu=x\pm t(s/n^{1/2})$, where s=standard deviation and n=number of replicates] that can be detected by the test value at the 95% confidence level. Confidence limits ranged from 0.02 to 0.15 m*M* depending on the sample studied.

4. Conclusions

The use of gradient anion-exchange HPLC with conductivity detection offers several advantages over previously published procedures for the determination of myo-inositol phosphates: (i) sample preparation is minimal: the lengthy purification step (1–2 days for 5–10 samples) is avoided and no derivatisation or precipitation is required. (ii) Evaporation of sample extract takes a shorter time (saving: 1 day) and the precision is improved by reducing the final solubilization volume (by 2/3). (iii) The retention time of phytic acid is only 6 min.

Comparison with other methods was difficult to perform because of lack of over data. However, as shown in Table 2, the sensitivity of ionic HPLC method with conductivity detection compared to reversed-phase HPLC method with refractive index detection makes the former useful for phytate analysis.

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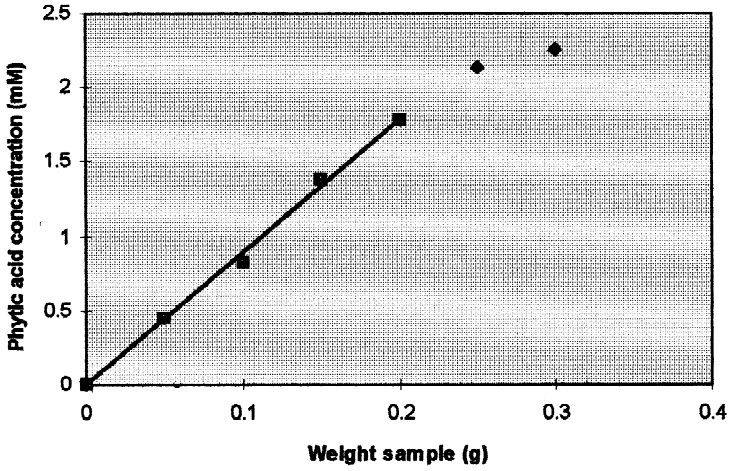

Fig. 3. Determination of optimum phytic acid concentration.

Table 2
Comparison of ionic HPLC analysis method with other HPLC analysis methods for the quantitation of phytic acid

	Sandberg and Ahderinne [13]	Matthaüs et al. [18]	Our proposed method
Number of replicates	6	10	6
Replicate analyses of a standard			
Average value (m*M*)	5.90		1.068
R.S.D.	2.0%	2.1%	0.56%
Limit of detection (μM)		0.056	0.0001
Replicate analyses of a food sample			
Sample nature	Textured soy flour	Rapeseed	Cowpea
Average value (μM/g)	21.8		8.4
R.S.D.[a]	5.7%	5.6%	3.6%

[a] R.S.D.=relative standard deviation.

References

[1] A.R. De Boland, G.B. Garner, B.L. O'Deel, J. Agric. Food Chem. 23 (1975) 1186.
[2] B. Nolan, P.A. Duffin, D.J. McWeeny, J. Sci. Food Agric. 40 (1987) 79.
[3] D. Oberleas, Cereal Foods World 28(6) (1983) 352.
[4] P. Besançon, in S. Trèche, B. de Benoist, D. Benbouzid, A. Verster and F. Delpeuch (Editors), L'Alimentation de Complément du Jeune Enfant, Orstom, Paris, 1995, p. 105.
[5] J.K. Chavan, S.S. Kadam, Crit. Rev. Food Sci. Nutr. 28 (1989) 401.
[6] J.K. Chavan, S.S. Kadam, Crit. Rev. Food Sci. Nutr. 28 (1989) 349.
[7] B. Lönnerdal, A.-S. Sandberg, B. Sandström, C. Kunz, J. Nutr. 119 (1989) 211.
[8] R. Holt, J. Sci. Food Agric. 6 (1955) 136.
[9] N.T. Davies, H. Reid, Br. J. Nutr. 41 (1979) 579.
[10] B. Tangendjaja, K.A. Buckle, M. Wootton, J. Chromatogr. 197 (1980) 274.
[11] E. Graf, F.R. Dintzis, Anal. Biochem. 119 (1982) 413.
[12] A.S. Sandberg, R. Ahderinne, J. Food Sci. 51 (1986) 547.
[13] C. Marquié, L. Segueilha, G. Moulin, V. Vialettes, P. Galzy, Coton Fibres Trop. 48 (1993) 87.
[14] M.A. Rounds, S.S. Nielsen, J. Chromatogr. A 653 (1993) 148.
[15] R.P. Scott, Liquid Chromatography Detectors, Elsevier, Amsterdam, 1977, p. 79.
[16] J. Caporal et al., S.T.P. Pharma 6 (1990) 588.
[17] D. Lecompte, S.T.P. Pharma 2 (1986) 843.
[18] B. Matthaüs, R. Loïsing, H.J. Fiebig, Fat Sci. Technol. 97 (1995) 289.
[19] J.C. Miller and J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood, Wiley, 1984, ISBN-085312-662-3.