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Note

Analysis of phytic acid by high-performance liquid chromatography

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Many workers[1–4] have determined phytic acid (inositol hexaphosphoric acid) in cereals by modifications to the original method of Heubner and Stadler[5], which depends upon the extraction of phytic acid from finely ground cereals and precipitation of phytic acid in the extract by addition of ferric chloride. A chromatographic method using a column containing an ion-exchange resin was first introduced by Smith and Clark[6] and has been used subsequently for the determination of phytic acid in soil[7] and blood[8,9]. The precipitation method, however, has been reported to be unreliable, and column chromatography cannot be used for routine analysis, since it is too time-consuming[10].

Recently, high-performance liquid chromatography (HPLC) has been attempted for the determination of phytic acid in pig manure. Separation, however, was unsatisfactory and prior chromatography of the extract on Sephadex was necessary. In the present investigation, a method is described for the HPLC determination of phytic acid in rice bran, and of inositol and inorganic phosphate as the end products of hydrolysis.

EXPERIMENTAL

Materials

Phytic acid solution (*ca.* 50% in water) was purchased from T.C.I. (Tokyo, Japan). Inositol (analytical-reagent grade) was obtained from Sigma (St. Louis, MO, U.S.A.) and sodium acetate (analytical-reagent grade) from Ajax Chemicals (Sydney, Australia). Acetonitrile was purchased from Waters Assoc. (Sydney, Australia) and water was double distilled in glass. Prior to use, all solvents were filtered through a 0.45-μm Millipore filter and degassed for 5 min. During degassing, solvents were shaken in an ultrasonic bath. Rice bran (variety Calrose) was obtained from the Ricegrowers' Cooperative Mills (Leeton, Australia).

Equipment

HPLC was carried out with Waters Assoc. (Milford, MA, U.S.A.) equipment consisting of a Model M6000A pump for solvent delivery, Model 440 ultraviolet (UV, 254 nm) and Model K401 differential refractive index (RI) detectors and a Model U6K injector. The Model 252 recorder was from Linear Instrument Corp. (Costamesa,

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CA, U.S.A.). μBondapak carbohydrate and C_{18} (30 cm × 4 mm) columns were obtained from Waters Assoc., and the syringes used for injection were from Hamilton (Reno, NV, U.S.A.).

Methods

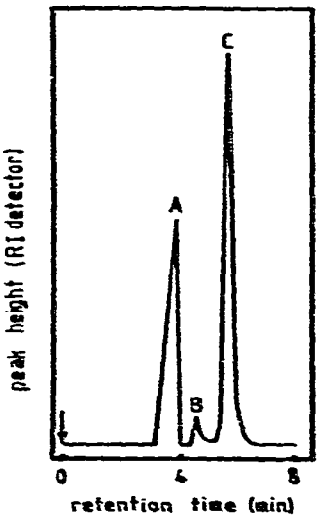
Aqueous solutions of phytic acid (0.5%, 10 μl) and inositol (1%, 5 μl) were mixed and injected onto a μBondapak C_{18} column. Sodium acetate (0.005 *M*) was used as solvent at a flow-rate of 0.5 ml/min; UV and RI detectors were used simultaneously.

Inositol and inorganic phosphate, obtained by heating 0.5% aqueous phytic acid solution for 48 h at 100°C and pH 4.3, were separated by injecting 25 μl on to a μBondapak carbohydrate column and developing with acetonitrile–water (60:40) at a flow-rate of 2 ml/min.

Phytic acid was extracted from rice bran (1 g) with 3% trichloroacetic acid (25 ml) for 30 min in a mechanical shaker, and the slurry was centrifuged for 20 min at 40,000 *g*. The supernatant liquid was filtered through a 0.22-μm Millipore filter, and an aliquot (25 μl) was injected into a μBondapak C_{18} column; the development solvent was sodium acetate (0.005 *M*) at a flow-rate of 2 ml/min.

RESULTS AND DISCUSSION

The ferric chloride precipitation method cannot be used for the determination of phytic acid in all foods and feeds because the presence of interfering substances such as reducing compounds leads to high results.



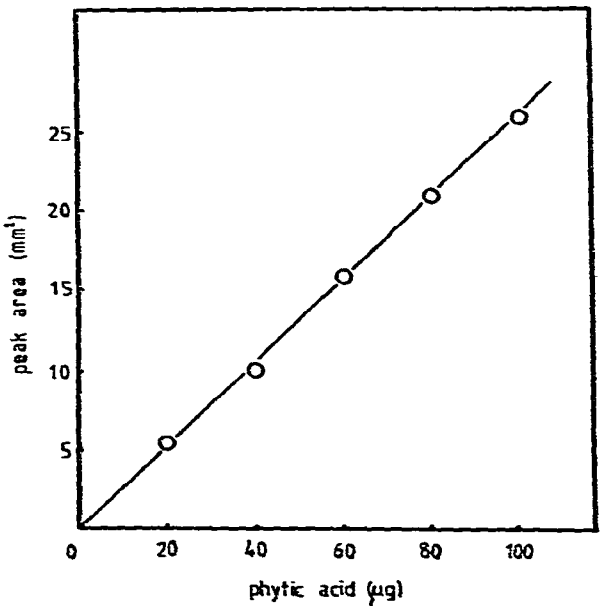
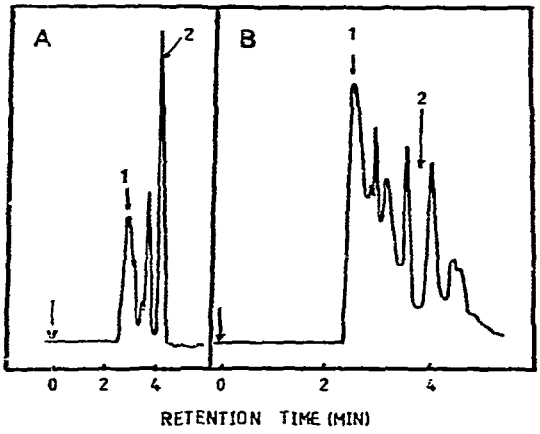


Fig. 1. Separation of phytic acid and inositol on a μBondapak C_{18} column developed with 0.005 *M* sodium acetate. Peaks: A = phytic acid; B = impurities from phytic acid; C = inositol.

Fig. 2. Standard curve for estimation of phytic acid.



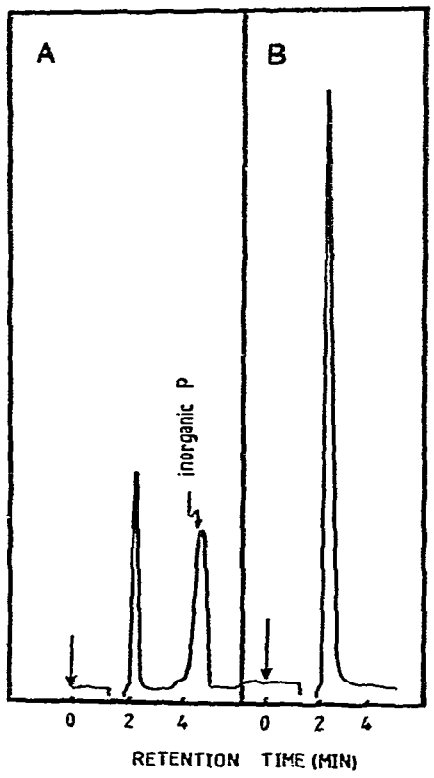


Fig. 3. Chromatogram of phytic acid solution after heating for 12 h at 100°C and pH 6.2. Separation was on a μBondapak C_{18} column developed with 0.005 *M* sodium acetate: A, RI detector; B, UV detector. Peaks: 1 = phytic acid; 2 = position for inositol.

Fig. 4. Chromatogram of phytic acid solution after heating for 48 h at 100°C and pH 4.3. Separation was on a μBondapak carbohydrate column developed with acetonitrile–water (60:40): A, hydrolysed phytic acid; B, inositol.

Ion-exchange chromatographic analysis[7] is not only time-consuming, but fails to detect inositol liberated from phytic acid during hydrolysis. Separation and estimation of both phytic acid and inositol is important for following the rate and extent of hydrolysis.

Fig. 1 shows the chromatogram of separation of phytic acid and inositol by HPLC on a μBondapak C_{18} column; separation was complete *ca.* 7 min after injection. The standard curve for phytic acid estimation is shown in Fig. 2.

The chromatogram of the hydrolysate of phytic acid produced by heating for 12 h at pH 6.2 is shown in Fig. 3. A UV detector gave more peaks than did the RI detector, but the former could not detect inositol. Inositol and inorganic phosphate, however, were separated on a μBondapak carbohydrate column with acetonitrile–water (60:40) as solvent (Fig. 4); increasing the acetonitrile concentration in the solvent gave better resolution, but broader peaks.

Phytic acid in rice bran can be estimated by separation on a μBondapak C_{18} column developed with 0.005 *M* sodium acetate (Fig. 5). Phytic acid was eluted from

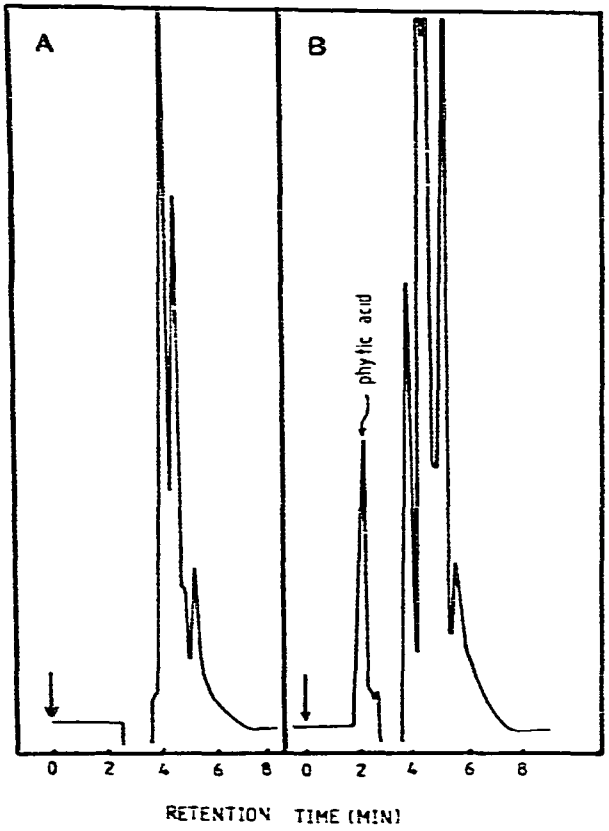


Fig. 5. Chromatogram of separation of phytic acid obtained from rice bran: A, trichloroacetic acid solution; B, rice-bran extract.

the column before trichloroacetic acid. Extraction of phytic acid with ethylenediaminetetraacetic acid solution as reported by Gerritse[11] requires prior separation by Sephadex gel chromatography before injection of the phytic acid extract on to a HPLC column.

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