INVESTIGATIONS ON PHYTIC ACID AND ITS DEGRADATION PRODUCTS IN RAPESEED AND RAPESEED MEAL

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ABSTRACT:

An HPLC method for the determination of phytic acid and three of its degradation products (IP3, IP4, IP5) in rapeseed and rapeseed meal has been developed. The method is based on ion pair chromatography with a RP-8-Phase and tetrabutylammoniumhydroxide as ion pair reagent. Methanol and bidestillated water (50:50) are used as mobile phase. The compounds are detected by a RI-detector. Variations in retention times and peak areas are avoided by thermostating the detector, the solvent reservoir and the column at 40 °C. The calibration of the system is achieved with pure phytic acid. An extraction method for inositol phosphates from rapeseed samples is optimized. The inositol phosphates of rapeseeds are extracted with 0.5 M HCl at 100 °C and cleaned up by an ion-exchange column. Degradation of inositolphosphates is not observed during this treatment. Different samples of rapeseeds and rapeseed meal are investigated and the results are presented. The results obtained by HPLC are compared with results of the method by Wheeler and Ferrel [1] and with those of a quick photometric method [2].

INTRODUCTION

Phytic acid [myo-inositol 1, 2, 3, 4, 5, 6 hexakis (dihydrogen phosphate)] is a naturally occurring component of most nuts, cereals, legumes and oilseeds, with amounts of 1 - 3 %. Because of the possible strong negative charge depending on the pH-value, phytic acid and its degradation products possesses an antinutritional potential depending on its phosphorylation. Hence the developement of methods for the determination of phytic acid is of great interest in the study of human and animal nutrition. In recent years some methods for the determination of phytic acid by HPLC have been developed [3, 4, 5, 6] to improve disadvantages of some classic methods [1].

The objective of this paper was to improve and modify the method of Sandberg [5] and to adapt this method on the determination of phytic acid and its degradation products IP3 - IP5 in rapeseed and rapeseed meal.

EXPERIMENTAL

Extraction

5 g of rapeseeds were crushed in a ball mill far10 min., then 0,3 g of the meal was defatted twice with 10 ml n-hexane in a lockable Pyrex vessel, dried at 50 °C, extracted with 25 ml 0.5 M hydrochloric acid for 10 min at 100 °C and centrifugated.

The phytic acid in the supernatant was separated by a modified ion exchange procedure of Graf and Dintzis [7]. 2 g of the resin was weighed into a glass column and flushed with 10 ml 2 N HCl, followed by 25 ml bidestillated water. The crude extract was passed through the column, the resin then washed with 30 ml 0.1 M NaCl and 25 ml bidestillated water. Phytic acid was eluated from resin with 30 ml 2 M HCl and the eluent removed by freeze-drying or evaporating to dryness at 40 °C and 50 mbar. The residue was dissolved in 1 ml bidestillated water.

HPLC

The HPLC was performed with a Merck-Hitachi 655-12 liquid chromatograph and L-5000 LC controller equiped with a Rheodyne model 7125 valve injector (10 µl loop) and fitted with a 125 x 4 mm, 5 µm LiChrospher 60 RP-select B column (Merck) used with a Merck type L-5025 column thermostated at 40 °C. Detection was carried out with a Merck RI-71 Differential Refractometer thermostated at 40 °C. The mobile phase consisted of bidestillated water/methanol (50:50) and 22.5 mmol/l TBA-OH was added. The pH was adjusted at 4.3 with 9 M sulphuric acid. The solvent reservoir was thermostated at 40 °C during the measurement. The flow rate was 1 ml/min Integration was performed with a Merck-Hitachi D-2000 Chromato-Integrator. Calibration and evaluation of the method were done with sodium phytate.

RESULTS

Extraction

Figure 1 shows the results of a plurally extraction of rapeseeds with 0,5 M HCl respectively bidestillated water at 100 °C. It demonstrates that the extraction of phytic acid with 0.5 M HCl is complete after the first extraction whereas the extraction with bidestillated water requires seven steps. A degradation of phytic acid by treatment with hot 0.5 M HCl was not observed.

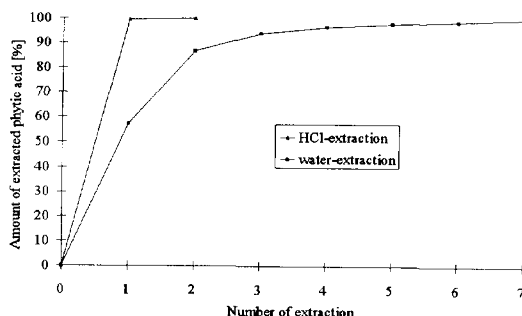

Fig. 1: Extractable phytic acid of a sample of rapeseeds depending on the number of extrations and the type of extracting agents (0.5 M HCl at 100 °C respectively bidestillated water at 100 °C,)

HPLC

The separation between inositol phosphates IP3 to IP6 was achieved by a short RP-8 phase within 8 min (Fig. 2).

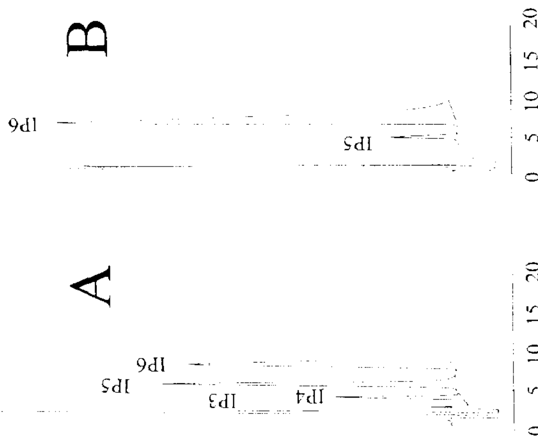

Fig 3: Chromatogram of (A) phytic acid standard solution and (B) a sample of rapeseeds

The limits of detection and determination are calculated according the DFG-Approach [8]. After calculating the calibration curve and the prognosisinterval the limits of detection and determination are evaluated graphically [9]. A seven-point-calibration was achieved twice with sodium phytate close to the limits of determination and detection. The limit of detection is at 0.26 mg/g seeds and the limit of determination at 0.39 mg/g seeds.

The variation coefficient was calculated with the values of ten injections of a solution of pure sodium phytate diluted in 0.5 M HCl and a sample of rapeseeds prepared ten times according 2.2. The values are at 11.6 % for IP5 respectively 5.6 % for IP6 determined in the sample of rapeseeds and 2.1 % for the solution of pure sodium phytate. The high variation coefficient for the determination of IP5 is explained by very small amount of it in rapeseeds.

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