INVESTIGATION OF RAPESEED PROTEIN-PHYTIC ACID COMPLEXES

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1. Introduction

Phytic acid, myo-inositol hexaphosphate, is one of the most important substances in rapeseed which can influence the properties of the protein owing to electrostatic interaction. Phytic acid and its protein complexes are known to decrease the availability of essential multivalent cations in the organism (MAGA, 1982). The high phytic acid content in rapeseed protein concentrates and isolates (APPELQUIST and OHLSON, 1972) necessitates the study of the influence of phytic acid not only on the nutritive properties but also on the behaviour of such protein preparations in food processing.
Solubility studies in rapeseed protein - phytic acid systems have been performed using protein extracts (GILBERG and TÖRNELL, 1976) and the purified 12 S globulin (SCHWENKE et al., 1979). Interaction studies in plant protein - phytic acid systems have been carried out with soybean (OKUBO et al., 1976) mustard (MURTHY and RAO, 1984) and rapeseed proteins (SCHWENKE et al., 1986, 1987). The present paper concerns a study of complex formation between phytic acid and the two main protein fractions from rapeseed, the high molecular mass 12 S globulin and the low molecular mass basic "albumin" fraction.

2. Results

The formation of insoluble complexes of phytic acid with both proteins has been observed in the pH-range 2 - 6, that means below the isoelectric points, using turbidimetric titration and chemical analysis of the supernatant after coprecipitation (SCHWENKE et al., 1986, 1987). It has been found that the amount of phytic acid bound to the proteins increased with decreasing pH. From the pH-dependence of phytic acid binding a "zero binding" in the isoelectric regions of both proteins was obtained (Figure 1). A (1 : 1)-stoichiometry of phytic acid binding to the globulin was found at pH 3.0, whereas only 0.8 mol phytic acid phosphate per mol basic groups were bound to the albumin at the precipitation point. The precipitation yields of the globulin and the albumin by interaction with phytic acid were 100 % and 80 % at pH 3.0.

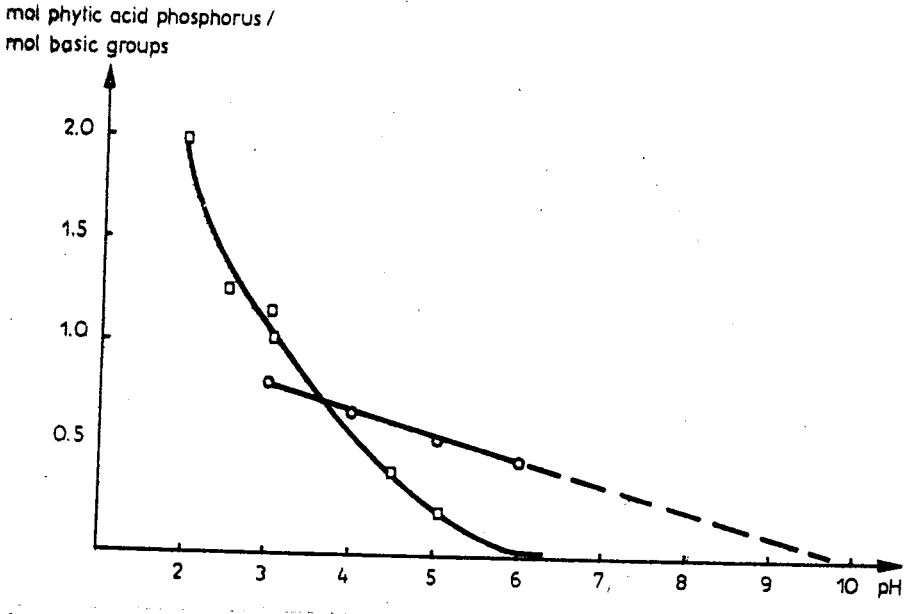

Figure 1. pH-dependence of phytic acid binding to globulin (□) and albumin (○).

This lower precipitation yield of the albumin is to be interpreted with regard to soluble phytic acid-protein complexes. The latter are formed as predominant reaction products at pH > 5 and are determined to be distinct protein oligomers. The gel chromatographic analysis revealed the doubling of the molecular mass of the protein from 14 000 to 28 000 g/mol in the presence of phytic acid in solutions of pH 6 - 8 (Figure 2). This protein dimer is stable in solutions of low and medium ionic strength ($\mu \leq 0{,}2$). The protein binds 0,08 g phytic acid per g under these conditions.
This phytic acid-protein ratio is a critical one to induce a stabilization against heat induced aggregation of the albumin. While phytic acid-free albumin solutions undergo an aggregation and coagulation on heating to temperatures > 50 °C, this heat-induced-aggregation is inhibited in the presence of phytic acid (c ≥ critical amount).

More quantitative results were obtained by means of quasi-elastic light scattering studies which allow to estimate the particle size distribution by the determination of the Stokes radii and scattering intensity of the aggregates. The relative scattering intensity of solutions of albumin-phytic acid complexes, related to the scattering intensity from monomeric albumin points clearly to the predominance of a dimerization product. Additionally, minor amounts of higher oligomers result from the size distributions. The essentially dimeric fraction has a mean Stokes' radius of 2.3 - 2.9 nm. This radius remains nearly constant after heating the solution to 60 °C. However, the percentage of the minor soluble subfraction with a Stokes' radius of 15 - 35 nm increases slightly. Contrary to this, in the absence of phytic acid, large aggregates are formed leading to a strong increase in the relative scattering intensity.

The secondary structure of the albumin which was determined by means of far ultraviolet circular dichroism spectroscopy and which is characterized by a high content (about 40 %) of α-helix conformation did not change upon interaction with phytic acid.

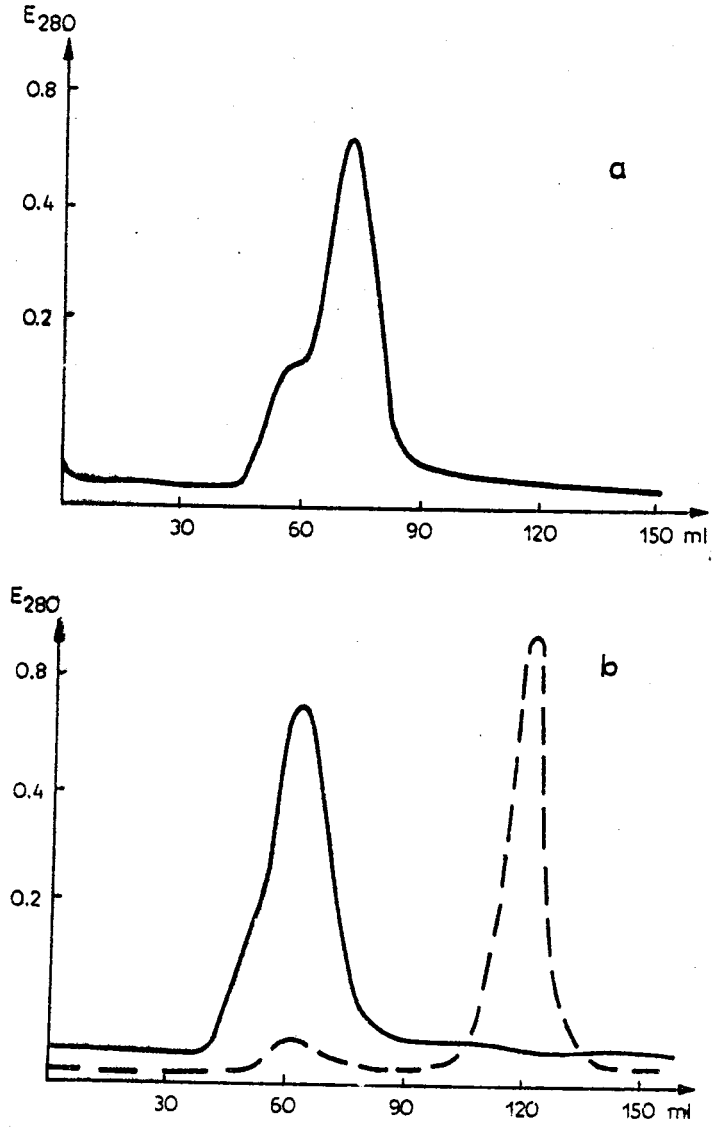

Figure 2. Gel chromatographic analysis of the albumin (a) and the albumin-phytic acid complex (b) on Sephadex G-50 in Tris-HCl buffer, pH 8,0, μ = 0.05

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