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PHYTIC ACID CONTENT OF CEREALS AND LEGUMES AND INTERACTION WITH PROTEINS

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Abstract

Phytic acid content of wheat, maize, barley, oats, soybean, cowpea, common bean, lupin and pea samples grown in Hungary were measured. In addition interaction of phytic acid with some isolated proteins has been studied. Legumes had a higher phytic acid content (max. 1.75 g/100 g) and the cereals a lower one (max. 1.42 g/100 g). The minimal value has been measured in durum wheat sample (0.52 g/100 g). Soy glycinin and sunflower seed globulin interacted with the highest quantity of phytic acid and gluten proteins with the lowest one. The protein–phytic acid interaction is highly dependent on pH.

Keywords: phytic acid, cereals, legumes, interaction, glycinin, protein isolates.

1. Introduction

Phytic acid (myoinositol, 1, 2, 3, 4, 5, 6 hexakis-dihydrogen phosphate) and phytate are widespread in plant seed grains (also including cereals), roots and tubers (LÁSZTITY and LÁSZTITY [1], GRAF [2]). Phytic acid is generally regarded as the primary storage form of both phosphate and inositol in seeds. Phytic acid phosphorus constitutes the major portion of total phosphorus in several seeds and grain. It accounts for 50–80% of the total phosphorus in different cereals. The phytic acid content in influenced by cultivar, climatic conditions and year. The accumulation site of phytic acid in monocotyledonous seeds (wheat, barley, rice, etc.) is the aleurone layer, particularly the aleurone grain. Aleurone grain contains two types of inclusions: (a) globoids containing high amount of phytates, and (b) protein carbohydrate bodies. Corn differs from other cereals as more than 80% of phytic acid is concentrated in germ. Phytic acid content of cereals varies from 0.5 to 2.0%.

Because most of the phytic acid is located in the outer parts of the kernel the different products of milling contain different levels of phytates. Bran is the product having a high phytic acid content, low extraction white flours contain low phytic acid quantities. If protein concentrates or isolates are prepared from cereals or other raw materials, such products contain also phytic acid in quantities depending on the raw material and method of processing.

The association of phytate with proteins begins in seeds during ripening, when phytate accumulates in the protein-rich aleurone layer of cereals and protein bodies

of legumes. Although the fine structure of phytate-rich particles in plants has been intensively studied, the nature of the interaction of proteins in such organelles with phytic acid is practically unknown.

The formation of globoid crystals and their size is highly dependent on the presence of inorganic cations. Higher amounts of magnesium and calcium favor the formation of large globoid crystals (GRAF [2], LOTT et al. [3]). This fact suggests that higher concentration of divalent cations increase phytate–phytic acid interactions rather than protein–phytic acid interactions. The conditions of processing such as addition of water, heat treatment (baking, autoclaving, extrusion, etc.), isolation and separation, action of phytate degrading enzymes (e.g. phytates, phosphomonoesters) may cause changes in the intensity and character of interactions.

This is a study of the possible phytic acid–protein interactions from theoretical point of view and the possibility of an electrostatic interaction in the protein–phytic acid system. Phytic acid molecule contains 12 dissociable hydrogens. Depending on the pH of the solution different phytic acid anions may be formed having different degree of protonation [4], [5].

On the other hand, protein molecules are also charged (except at the pH corresponding to the isoelectric point). Several studies indicate the involvement of some side chains of proteins in the formation of protein–phytic acid complexes. The results of such studies are summarized in reviews by REDDY et al. [6] and CHERYAN [7]. At a low pH, below the isoelectric point of proteins, the terminal amino, lysyl, histidyl and arginyl groups can be positively charged. Any of these groups can directly form a complex with a negatively charged phytate anion. If the steric conditions are satisfactory, one phytate anion can interact with two charged groups of protein. Naturally, the protein molecule can bind more phytate anions at the same time, depending on the number of positively charged groups and conformational conditions. At intermediate pH values, only the lysyl and arginyl groups are positively charged, so in this case a slight possibility of electrostatic interactions exists. If the pH is very high, the interaction between phytic acid and protein is diminished.

The presence of other components in the food system gives additional possibilities for protein–phytic acid interactions. If polyvalent cations are present, ternary complexes can be formed. In this case the cation forms a bridge between the phytate anion and a negatively charged group of the protein, which allows the binding of phytic acid by proteins at neutral and high pH. Investigations by THOMPSON [8] showed that other types of ternary complexes can also occur. It was suggested that ternary complexes of protein, phytic acid and carbohydrate (starch) might form, which subsequently affects the digestion rate of starch.

There have been no reports on the existence of ternary groups of protein, lipid and phytic acid, although the occurrence of such interactions cannot theoretically be excluded.

The knowledge of possible interaction sites provides some clue to influence the interaction of proteins with phytic acid. As tools in such a procedure, one could use the pH, the quality and quantity of ions in the system, and chemical or enzymatic modification of the surface groups of proteins.

The aim of work in our laboratory was the study of phytic acid content of some cereals and legumes grown in Hungary and also of some concentrates and isolates, the study of interactions of phytic acid with some proteins and finally the effect of phytic acid content on functional properties of protein isolates and concentrates.

2. Materials and Methods

Three wheat varieties (MV-4, Besostaya-1, GK-Basa), three maize cultivars (yellow dent, flint and sweet corn), barley, oats, soybean, cowpea, common bean, lupine and peas samples grown in Hungary were used.

Commercial soy isolate and soy concentrate, as well as vital gluten was used in experiments, lupine seed protein isolate was prepared by alcoholic extraction, acid precipitation, dialysis against water and freeze drying.

For model experiments soy glycinin (OKUBO et al. [9]), sunflower seed globulin (LÁSZTITY et al. [10]) and gluten protein preparations were used. Full gluten was prepared after defatting of flour and washing the dough with distilled water. Gliadin was extracted from gluten with 70 % ethanol and acetic acid soluble glutenin was prepared from the residue with 0.05 M acetic acid. All the three preparations were dissolved in sodium hydroxide (0.1 M NaOH) and ultrafiltrated to remove phytic acid from the preparations.

3. Results and Discussion

The phytic acid content of investigated samples (5–5 samples from two years crop) is shown in *Table 1*. The phytic acid content and protein content of isolates and concentrates is shown in *Table 2*. Soy isolates had the lowest phytic acid content and the vital gluten the highest one.

Glycinin reacted with phytic acid strongly below pH 5. At pH values above the isolelectric point (pH 6, 5 and 12), no binding of phytic acid was observed. Decreased solubility and precipitation were observed in the pH range 2.0–5.5. The maximum bound quantity of phytate was 126 mol per 10^5 g of protein. This agrees well with the number of cationic groups in glycinin (calculated on the basis of amino acid composition) and with data published by OKUBO et al. [11].

Sunflower seed globulin was similar to soy proteins in the binding of phytic acid. This similarity may be associated with the similar solubility of the proteins from the two sources and with some similarities in their amino acid composition. The isolelectric point was somewhat lower (pH 4.5) than that of glycinin. Phytic acid binding occurred only below the isoelectric point. The highest amount bound was lower (90 equivalents of phytic acid per 10^5 g of protein) than that in the case of glycinin.

The binding of phytic acid by gluten proteins was much lower than that of soy and sunflower globulins, which may be related to the lower number of cationic

Table 1. Phytic acid content of some cereals and legumes (g/100 g), (5–5 samples from two years crop)

Product	Phytic acid content*		
	Average	Minimum	Maximum
Wheat (MV-4)	0.85	0.67	0.98
Wheat (Besostaya-19)	0.93	0.75	1.05
Wheat (durum, GK Basa)	0.72	0.52	0.78
Maize (yellow dent)	1.02	0.80	1.17
Maize (flint)	0.90	0.78	1.02
Maize (sweet)	0.85	0.62	1.06
Barley	0.97	0.85	1.18
Oats	1.01	0.90	1.42
Soybean	1.43	1.20	1.75
Cowpea	0.42	0.29	0.86
Common bean	0.55	0.90	1.69
Lupine	1.38	0.76	1.63
Peas	1.02	0.72	1.23

*Calculated phytic acid content assuming 28.20 % phosphorus in the molecule

groups, especially in gliadin components. The maximum value of bound phytic acid was only 30 equivalents of phytic acid per 10^5 g of protein.

Table 2. Protein and phytic acid content of some concentrates and isolates (g/100 g)

Protein preparation	Protein content	Phytic acid content*
Soy isolate	90.8	0.82
Soy concentrate	69.2	1.01
Vital gluten	80.1	1.90
Lupine seed protein isolate	74.2	1.43

* Calculated phytic acid content assuming 28.20% phosphorus in the molecule

Investigating gliadin solutions in acidic pH region the maximum binding of phytic acid was observed in pH range 2.5–3.5. At pH-s lower than 2.5 a rapid decrease was observed and at higher pH values a stepwise one. This character of changes may well be explained with the dissociation (or protonation) of phytic acid. At very low pH values the phytic acid does not practically have charges and so could not electrostatically react with proteins. At higher pH values the limiting factor of electrostatic interaction is the decreasing charge on the protein molecules.

It was reported by some authors (SCHWENKE et al. [12], RAJENDRAN and PRAKASH [13]) that interaction with phytic acid is connected with aggregation of proteins, which was confirmed by changes of elution profiles on Sephadex columns after binding of phytic acid by proteins. We were not able to detect such aggregation. This may be connected with the fact that at lower pH values not all of the phosphate groups are dissociated. Phytic acid level has no or very little effect on binding to proteins.

The investigation of the possibility of formation of ternary complexes raises difficulties. At alkaline pH values the Ca-phytate is insoluble and forms precipitate. At very high pH values the phytate is insoluble. It must also be noted that the gluten, gliadin and glutenin preparations always contain some calcium, magnesium and zinc (LÁSZTITY et al. [14]).

As mentioned, functional properties of soy concentrates, soy isolates and vital gluten with increased (3.0 %) phytic acid content were prepared by phytic acid addition and the functional properties were compared with those not treated with additional phytic acid. Nitrogen solubility, water absorption, emulsifying activity, emulsion stability and last gelation concentration were determined using methods of DEV and MUKHERJEE [15]. No significant changes were observed. Thus, lowering the phytate content in protein preparations may be important primarily from a nutritional point of view. These conclusions are in agreement with the results of experiments made by NACZK et al. [16].

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