Phytic Acid in Faba Bean and Pea: Effect on Protein Availability

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ABSTRACT

Several cultivars of faba bean and pea and their protein products obtained by different methods were studied to determine the interaction of phytic acid with protein and its effect on protein availability. Data showed that binding between phytic acid and protein was not affected by physical methods of separation (dehulling, air classification); the weight ratio of phytic acid to protein remained constant (1:29) in different fractions of the same source. In vitro digestibility of faba bean and pea samples decreased in the presence of exogenous phytic acid; a reduction of 6.8, 5.7, and 8.7%, respectively ($P < 0.001$) occurred in whole flour, protein concentrate, and protein isolate following the addition of 10 mg of phytic acid. A reduction in in vitro protein digestibility of five protein sources (lactalbumin, casein, serum albumin, zein, and soy protein isolate) occurred when 1, 3, 5, or 10 mg of phytic acid was added. The lowest reduction of digestibility was observed for lactalbumin (3.8%) and the highest for zein (11.1%). These data support the hypothesis that phytic acid-protein interaction affects the protein availability of legumes negatively and that the nature of the protein source plays a prominent role.

The availability of nutrients in plant foods may be affected by natural complexing agents. This is particularly true in the case of seeds containing phytic acid, such as legumes, cereals, and oilseeds. In fact, because of its highly reactive structure, at different pH levels phytic acid can complex proteins as well as mono- and divalent cations. Phytic acid and its chemical and nutritional effects have been extensively reviewed (Cheryan 1980, Reddy et al 1982). From a nutritional point of view, many studies have concentrated on the metal ion chelating property of phytic acid (Erdman 1981), its binding of zinc and formation of less soluble complexes that reduce zinc availability (Prasad 1979, Morris and Ellis 1980).

The interaction of phytic acid with protein has been studied mainly in soy beans. Such studies describe the formation of phytate protein complexes, their effects on protein solubility and related properties, and methods for phytic acid removal (Okubo et al 1975, 1976; O'Dell and DeBoland 1976; De Rham and Jost 1979; Omosaiye and Cheryan 1979; Honig et al 1984). However, the nature of the phytate-protein interaction is not completely understood, and its nutritional effects on protein availability still need clarification. Phytate-protein binding is affected by several factors, such as the characteristics of the protein matrix. The purpose of the present investigation was to determine the effects of phytic acid-protein interactions in several cultivars of faba bean and pea on in vitro protein digestibility.

MATERIALS AND METHODS

The primary faba bean cultivar used in this study was Vesuvio. Other cultivars of faba bean (Manfredini, Polo, and Aguadulce) and pea (Finale, Imposant, and Rondo) were also studied in some cases.

Seeds were mechanically dehulled; whole seeds, hulls, and cotyledons were ground in a laboratory mill to pass a size <50 mesh screen.

Protein concentrates were prepared from whole seeds (cultivars Vesuvio, Imposant, Finale) and dehulled seeds (Manfredini) utilizing an Alpine pilot plant (pin mill Kolloplex 160Z and air classifier Mikroplex 132 MP) using the conditions reported by Carnovale and Cappelloni (1983).

Protein isolate was prepared from the Vesuvio faba bean protein concentrate by extraction for 30 min at pH 7.0 (solid-liquid ratio 1:20), precipitation at pH 4.8, centrifugation, washing at pH 4.8, and protein recovery by centrifugation and drying (Albonico, in press).

Determination of Phytic Acid and Phosphorus

In all samples, the method of Harland and Oberleas (1977) for determination of phytic acid was used, with a minor modification, i.e., the sample was extracted with 2.4% HCl, and its dilution was reduced to 1:5.

Phosphorus was determined using the molybdovanadate method of the AOAC (1984).

Determination of Solubility of Phytic Acid and Protein

The solubility of protein from faba bean (Vesuvio) flour as a function of pH, was determined according to the method of Hermansson (1972). The sample was dispersed in water and the pH adjusted to the desired value with 0.1*N* NaOH and 0.1*N* HCl. The suspension was stirred for 45 min at room temperature, and after centrifugation at 40,000 × *g*, aliquots of the supernatant were analyzed for protein (N × 6.25) according to the AOAC method (1980), and phytic acid was determined as described above.

Diafiltration

Flour and protein concentrate from Vesuvio faba beans were suspended in distilled water and filtered (Amicon ultrafilter, Diaflo H1P320 membrane with nominal cutoff of 3,000 Da) until the final volume reached four times the initial volume. The diafiltrate and the residue were freeze-dried, and the phytic acid content was determined in both.

Ultrafiltration

Ultrafiltration was performed on both flour and protein concentrate obtained from cultivar Vesuvio. Samples were suspended in distilled water with the pH adjusted to 7.0 and then ultrafiltered (Amicon model 52 equipped with a YM2 membrane, nominal cutoff of 1,000 Da) at 4 atm for 24 hr. The permeate and

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the retentate were then freeze-dried, and the phytic acid content was determined in both.

In Vitro Protein Digestibility

In vitro protein digestibility was estimated according to the multienzymic method of Hsu et al (1977) as modified by Satterlee et al (1979).

The following procedure was used to test the effect of phytate on protein digestibility: the sample, providing 10 mg of N, was dissolved in solutions containing 1, 2, 3, 5, or 10 mg of sodium phytate (Sigma) and left for 1 hr at room temperature. Determinations of digestibility were then made according to the method described above.

RESULTS AND DISCUSSION

The protein and phytic acid contents of the samples examined are shown in Table I. Phytic acid content in faba bean seeds ranged from 0.71 to 1.15% and from 0.75 to 0.94% for peas. Phytic acid was predominantly located in the cotyledons; the hulls of faba bean contained from 0.06 to 0.20% of the phytic acid. Air classification greatly concentrated the phytic acid in the protein fraction; it more than doubled with respect to the starting whole seed flours. These data agree with the results of Vose (1976).

Dehulling and air classification methods concentrated the protein, and phytic acid content increased in proportion to the increase in protein content. The weight ratio between these two constituents remained constant at an average of 1:29. Protein and phytic acid contents showed a high correlation coefficient ($r = 0.99$, $n = 30$), suggesting that phytic acid is bound to the protein component of the seed, presumably the protein bodies.

The removal of phytic acid from Vesuvio flour and protein concentrate using diafiltration and ultrafiltration tests was studied. Under the experimental conditions adopted (ultrafiltration for 24 hr and diafiltration to a volume four times the initial volume), phytate was not removed from either product. The data suggest that a relatively stable bond exists between phytic acid and protein. Other literature (Okubo et al 1975, Omosaiye and Cheryan 1979) reports difficulties in the separation of protein from phytic acid in soybeans using ultrafiltration. The authors obtained a reduction in phytic acid content only by using successive protein fractionations under strictly controlled pH conditions.

Because the solubility of phytic acid and protein is different for different protein sources and at different pH levels (Kanta et al 1986), we studied solubility profiles in faba bean. The solubility profile in Figure 1 shows that at acidic pH protein and phytic acid curves overlap, showing a minimum at pH 3.5. Solubility of phytic acid at higher pH levels is greater than that of protein, especially at pH values between 4 and 6. An analogous profile of solubility was observed in soybean (Cheryan 1980).

The phytic acid content of a faba bean protein isolate prepared according to Albonico (in press) was found to be 2.41% (Table I), corresponding to 40% of the starting content and a protein-to-phytic acid ratio of 39. Phytic acid was consistently found in all faba bean and pea protein products assayed. It was virtually impossible to remove all of the phytic acid by physical processes. A reduction in phytic acid greater than 60% could be reached using solubilization methods. This, however, was not satisfactory because of the low protein recovery and the numerous purification steps required.

Because faba bean and pea can be an important source of protein (CNR 1979), we studied the effect of phytic acid on protein availability. Protein digestibility was determined to provide the most satisfactory indication of protein utilization (FAO/WHO/UNU 1985). Data on in vitro protein digestibility of representative faba bean and pea samples are reported in Figure 2. The digestibility of flour from whole and dehulled Vesuvio seed and its protein concentrate were very similar, 84.3, 84.9, and 83.9, respectively. Vesuvio protein isolate, however, showed a

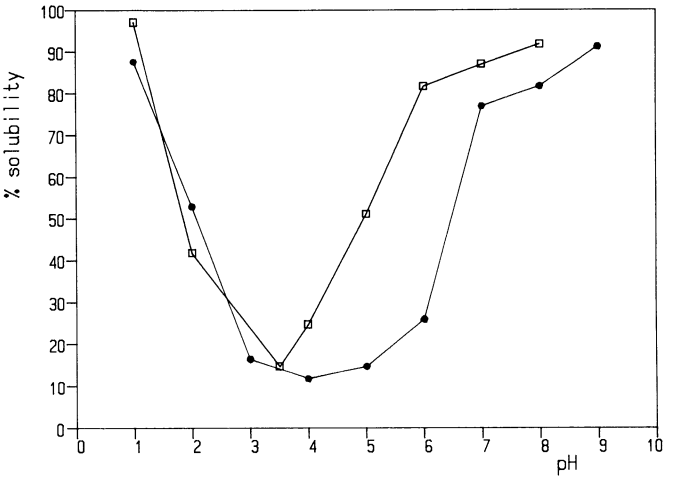

Fig. 1. pH solubility profile of phytic acid (□) and nitrogen (•) in flour (whole seed) from faba bean cultivar Vesuvio. Each value is the mean of three determinations.

TABLE I
Moisture, Protein, and Phytic Acid Content of Faba Bean and Pea Samples (% dry weight basis)

Sample/ Cultivar	Part	Water[a]	Protein[a] (N × 6.25)	Phytic Acid
Faba bean (*Vicia faba*)				
Vesuvio	whole seed	10.3 ± 2.1	28.0 ± 0.1	0.86 ± 0.06
	hulls	7.4 ± 0.8	4.6 ± 0.8	0.20 ± 0.10
	dehulled seed	10.7 ± 1.4	32.9 ± 1.1	1.16 ± 0.09
	protein concentrate (from whole seed)	8.7 ± 0.1	60.9 ± 0.4	2.10 ± 0.10
	protein isolate	2.1 ± 0.1	95.1 ± 0.4	2.41 ± 0.19
Manfredini	whole seed	10.9 ± 0.9	24.0 ± 0.2	0.87 ± 0.01
	hulls	6.9 ± 0.6	3.7 ± 1.1	0.06 ± 0.09
	dehulled seed	12.5 ± 0.1	27.4 ± 0.1	0.95 ± 0.02
	protein concentrate (from dehulled seed)	8.8 ± 0.2	60.8 ± 1.5	2.07 ± 0.01
Polo	whole seed	10.9 ± 1.0	27.3 ± 1.6	1.15 ± 0.07
Aguadulce	whole seed	10.2 ± 0.8	23.9 ± 0.8	0.71 ± 0.01
Pea (*Pisum sativum*)				
Imposant	whole seed	10.2 ± 1.2	22.3 ± 0.4	0.85 ± 0.01
	protein concentrate	9.8 ± 0.1	50.0 ± 0.4	1.90 ± 0.02
Finale	whole seed	11.1 ± 1.2	22.4 ± 0.3	0.94 ± 0.07
	protein concentrate	8.1 ± 0.8	35.3 ± 0.9	1.32 ± 0.14
Rondo	whole seed	10.3 ± 0.1	23.5 ± 0.4	0.75 ± 0.07

[a] Mean ± SD; each value is the mean of three determinations.

significantly higher digestibility (91.6%). Manfredini dehulled seeds and protein concentrate and Imposant protein concentrate showed digestibilities similar to the corresponding Vesuvio samples.

Protein isolate had the highest protein-phytic acid ratio and showed the highest digestiblility. In contrast, Imposant protein concentrate had the lowest ratio and the lowest digestibility. It is difficult to clearly define the effect of endogenous phytic acid on protein digestibility, since antitryptic factors, tannins, and fiber also present in the samples affect protein digestibility (Carnovale et al 1983).

Exogenous phytic acid (5 mg/sample) was added to the samples in order to clarify the effect of phytic acid on digestibility. This amount doubled or trebled the natural phytic acid content of the samples. Although digestibility was reduced in all samples with added phytic acid (Fig. 2), the differences were not statistically significant. Protein digestibility was also determined on whole flour and protein concentrate obtained by dry processes and protein isolate obtained by wet processes after addition of 1, 2, 3, 5, and 10 mg of phytic acid. Protein digestibility profiles of the three samples were similar (Fig. 3). However, in the isolate, the decrease in digestibility due to increased phytic acid level was more pronounced at each level. When 10 mg of phytic acid was added, corresponding to a phytic acid-protein ratio of 1:5, the reduction in digestibility was significant ($P < 0.001$) in all three samples. Reductions of 6.8, 5.7, and 8.8%, respectively, for flour, protein concentrate, and isolate, were observed.

These results, showing a negative correlation between phytic acid content and in vitro protein digestibility, agree with the data of other workers (Knuckles et al 1985, O'Dell and DeBoland 1976, Serraino et al 1985).

Anderson (1985) reviewed the interaction between phytic acid and protein and reported conflicting results in in vivo experiments with regard to phytate intake and protein digestibility. The reason for these discrepancies could lie in the nature of the protein sources. To clarify this point, the in vitro protein digestibility of five protein sources: lactalbumin, casein, bovine serum albumin, zein, and soy protein isolate, before and after addition of 1, 3, 5, and 10 mg of phytic acid was tested. The initial digestibilities were, in order, 84.3, 91.3, 93.8, 88.9, and 92.0 (Fig. 4). The responses differed with the sources of protein, each protein showing a particular profile. Lactalbumin showed the lowest and zein the highest response. The reduction in protein digestibility after adding 10 mg of phytic acid was 3.8% for lactalbumin, 7.6% for casein and soy protein isolate, 8.0% for serum albumin, and 11.1% for zein.

Differences in phytic acid-protein binding are difficult to explain. Differences in buffering capacity of protein do not seem to be responsible. The work of Knuckles et al (1985) showed less effect of phytate on digestion of bovin serum albumin than on casein using a different in vitro procedure to measure digestibility (determination of nitrogen in dialysate of pepsin digestion).

From our preliminary results, the content of free NH_2 groups does not seem to correlate with variations in digestibility. This agrees with the findings of O'Dell and De Boland (1976), who studied phytate-protein interaction in corn germ and oilseeds.

Faba bean protein isolates show the same profile of digestibility reduction as soybean. Because chemical and biochemical protein characteristics of soybean and faba bean are very similar, this again suggests that the effect of phytic acid is closely related to the

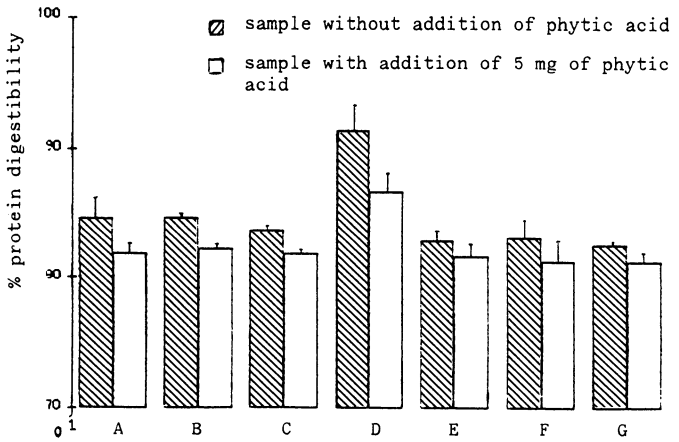

Fig. 2. In vitro protein digestibility of faba bean and pea samples with and without the addition of 5 mg of phytic acid. Cultivar Vesuvio: **A,** whole seed; **B,** dehulled seed; **C,** protein concentrate; **D,** protein isolate. Cultivar Manfredini: **E,** dehulled seed; **F,** protein concentrate. Cultivar Imposant: **G,** protein concentrate. Mean of four determinations ± standard deviation.

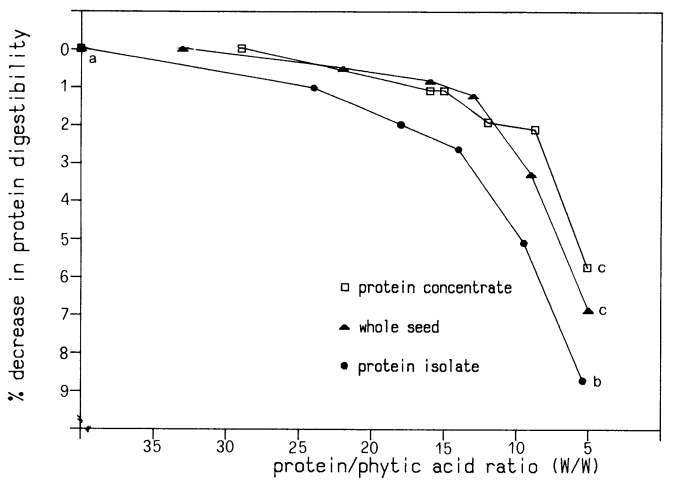

Fig. 3. Effect of increasing amounts of phytic acid on in vitro protein digestibility of faba bean samples. Phytic acid content (naturally present plus 1, 2, 3, 5, or 10 mg) is related to protein content. Different letters denote a significant ($P < 0.001$) difference. Each value is the mean of three determinations.

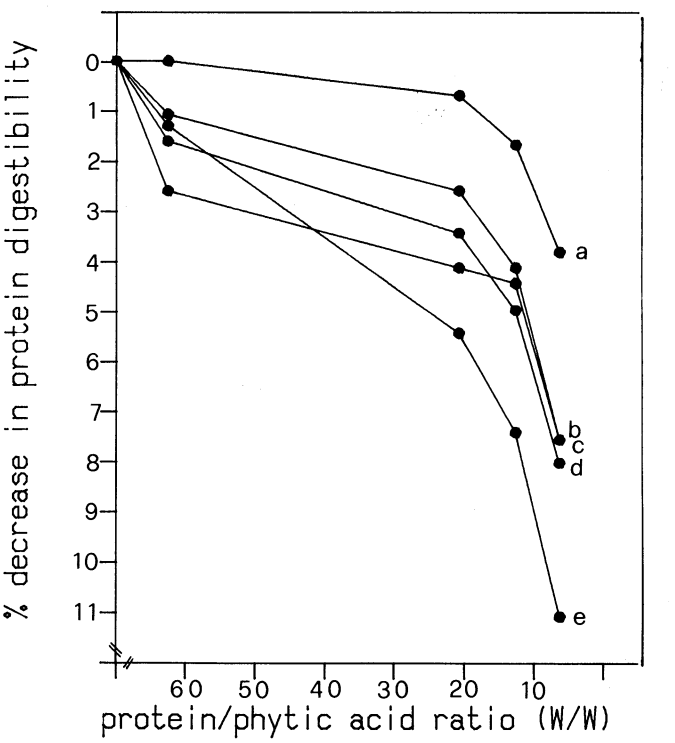

Fig. 4. Effect of increasing amounts of phytic acid on in vitro protein digestibility of protein samples. **a,** Lactalbumin; **b,** soybean protein isolate; **c,** serum albumin; **d,** casein; **e,** zein. Phytic acid was added at 1, 3, 5, and 10 mg. Each value is the mean of three determinations. At 10 mg of phytic acid addition, for a versus e, $P < 0.01$; for a,e versus b,c,d, $P < 0.05$.

protein matrix. Further studies are necessary to clarify the mechanism of binding.

Our results indicate that removal of phytic acid from faba bean and pea protein is difficult, and therefore, phytic acid is present in commercial faba bean and pea products and consequently may affect their technological and nutritional properties. Even though the reduction in mineral availability is the main nutritional effect of phytic acid, the nutritional and technological consequences of its effects on protein digestibility must also be taken into account.

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