Removal of Phytic Acid and Protein–Phytic Acid Interactions in Rapeseed

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The effect of pH on the disruption of protein–phytate complexes, and hence on the removal of phytic acid from rapeseed flour (RF) and protein concentrate (RPC) in a dialysis system, was examined. Phytic acid removal from RF and RPC dispersions was also tried by using various amounts of EDTA (0.25–0.75 M) at pH 9.0, $CaCl_2$ (0.25–0.75 M) at pH 3.5, or phytase (3%) at pH 5.15. Dispersions of RF and RPC were dialyzed against distilled water for 7 days. While the greatest removal of phytic acid (100%) with the least loss of nitrogen was observed with phytase treatment, mere adjustment of RPC to pH 3.5 and of RF to pH 5.15 resulted in 73% and 88% losses of phytic acid, respectively. Dialysis of RF dispersion without pH preadjustment, i.e., at pH 5.8, removed up to 67% of the phytic acid. The success of the method in reducing phytic acid levels in rapeseed provided insight as to the nature of the protein–phytate interactions present in rapeseed. Nitrogen losses were not greatly influenced by pH adjustment but were enhanced by the other treatments applied. These losses, however, never greatly exceeded 30% with RF and 18% with RPC.

Rapeseed is a major oilseed crop in Canada. The seed contains about 40% oil and approximately 25% protein, while the defatted meal has as high as 40% protein. This protein has a well-balanced amino acid composition and is especially rich in lysine and sulfur-containing amino acids (Gillberg and Tornell, 1976); thus, it seems suitable for complimenting cereals and many legumes that are deficient in those amino acids.

The utilization of rapeseed protein has been limited by the presence of many antinutrients and toxic constituents associated with the protein fraction. Among those is phytic acid [*myo*-inositol 1,2,3,4,5,6-hexakis(dihydrogen phosphate)], which is present in rapeseed at levels as high as 5–7% (Shah et al., 1976). Phytic acid is strongly negatively charged over most of the pH scale, suggesting a tremendous potential for complexing positively charged molecules such as cations or proteins with the formation of complexes that may be insoluble or otherwise unavailable under physiologic conditions (Cheryan, 1980). This leads to a decrease in the bioavailability of many essential minerals, especially zinc (Erdman, 1979). In addition, complex formation of phytic acid with proteins obstructs the enzymatic degradation of the protein (O'Dell and de Boland, 1976). Hence, methods for reducing the phytic acid content of rapeseed by disrupting the protein–phytate complex are being examined.

The extent of phytate–protein binding depends on both pH and/or the content of divalent ions. At acid pH, the proteins possess a net positive charge while phytic acid is negatively charged; thus a binary protein–phytate complex forms. Excess Ca ion is believed to dissociate this complex by competing for phytate with the cationic groups of protein (Cheryan, 1980). At alkaline pH, both protein and phytic acid are negatively charged; thus the interaction is mediated by multivalent cations to form a ternary protein–mineral–phytate complex. Disruption of this complex requires the removal of cations from the system by use of chelating agents such as ethylenediaminetetraacetic acid (EDTA), to which some cations will bind more preferentially than to phytate. Soluble cation–EDTA complexes are formed, thus preventing the formation of the protein–phytate complex.

The objective of this study was to examine the effects of pH adjustment on the protein–phytate complex and hence on removal of phytic acid from rapeseed flour (RF)

and protein concentrate (RPC) in a dialysis system. In order to improve the phytate removal and, also, to gain some understanding as to the nature of the protein–phytate complex present in rapeseed, the effect of excess $CaCl_2$ at acid pH, excess EDTA at alkaline pH, and phytase treatments at pH 5.15 was also determined.

MATERIALS AND METHODS

Dehulled, solvent-extracted, non-heat-treated RF and FR1-71 RPC processed according to the procedure of Tape et al. (1970) were provided by Dr. J. D. Jones, Food Research Institute, Ottawa, Canada. EDTA, anhydrous $CaCl_2$, and crude wheat phytase were purchased from Fisher Scientific Co., Baker Chemical Co., and Sigma Chemical Co., respectively.

Five percent (w/v) dispersions of RF or RPC were prepared by using distilled water and treated with either one of the following: (a) The pH was adjusted to either 3.5 or 5.15 by using 6 N HCl or 9.0 by using 6 N NaOH. Dispersions that were not pH adjusted served as controls and had a pH of 5.8. (b) One of three concentrations of EDTA (0.25, 0.50, or 0.75 M) was added and the pH was then adjusted to 9.0 with 6 N NaOH. (c) One of three concentrations of $CaCl_2$ (0.25, 0.50, or 0.75 M) was added and the pH was then adjusted to 3.5 by the addition of 6 N HCl. (d) Wheat phytase (3%) was added at pH 5.15, followed by a 2-h incubation period at 57 °C.

All dispersions were dialyzed (Spectrapor membrane tubing, molecular weight cutoff 6000–8000) for 7 days against distilled water, which was changed twice daily. Dialysis retentates were freeze-dried, weighed to determine recovery, and analyzed for phytic acid by using the method of Latta and Eskin (1980) and nitrogen by micro-Kjeldahl (AOAC, 1980).

Phytic acid and nitrogen losses were calculated as

$$\% \text{ loss} = (AB - CD)/AB \times 100 \qquad (1)$$

where A = weight of the undialyzed sample, B = percent phytic acid or nitrogen of the undialyzed sample, C = weight of the dialyzed sample, and D = percent phytic acid or nitrogen of the dialyzed sample.

The reported values represent averages of at least four determinations, each obtained as duplicate analyses of at least two replicate dialyses.

RESULTS AND DISCUSSION

Phytic Acid Losses. To test the effect of pH on phytic acid loss in RF or RPC, sample dispersions were adjusted from its initial pH of 5.8 to either pH 3.5, 5.15, or 9.0 prior to dialysis. Those pHs were chosen based on the solu-

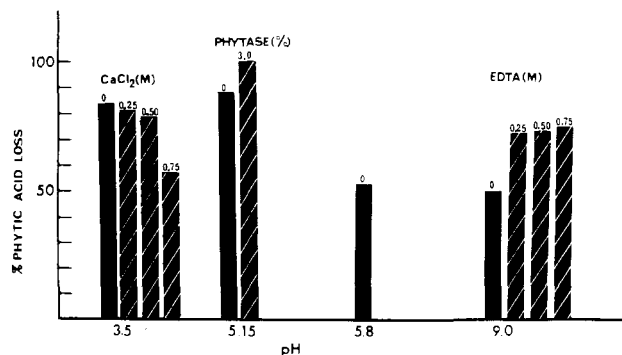Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Figure 1. Effect of pH adjustment, EDTA, CaCl₂, and phytase enzyme treatment on phytic acid removal in rapeseed flour.
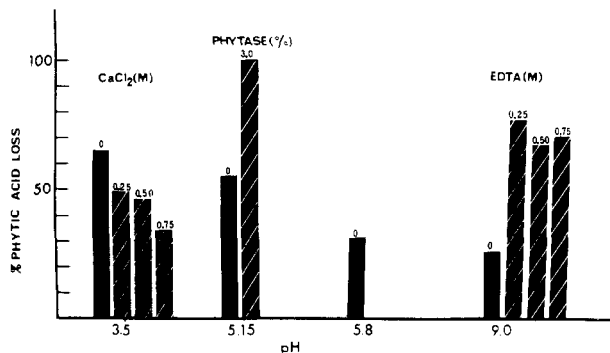

Figure 2. Effect of pH adjustment, EDTA, CaCl₂, and phytase enzyme treatment on phytic acid removal in rapeseed protein concentrate.

bilities of phytic acid and protein (Gillberg and Tornell, 1976). The pHs that minimize nitrogen solubility while maximizing phytic acid solubility are desirable for dialysis, for, under such conditions, there is less likely to be any interaction between the protein and phytate. On the acid side of the pH scale, pH 3.5 was chosen because this pH is just below the isoelectric pH of the proteins present in rapeseed while phytic acid is still soluble. The pH 5.15 was used as this is the pH at which the Ca and Mg salts of phytic acid show maximum solubility while nitrogen solubility is relatively low. The alkaline pH 9.0 was chosen to see if any loss of phytate would occur. A pH higher than 9.0 was not studied as, at more alkaline conditions, rapeseed products acquire a dark color and strong flavor.

The phytic acid losses obtained for RF and RPC are shown in Figures 1 and 2, respectively. Zero treatment levels were used in evaluating the pH effects upon phytate losses in RF and RPC samples. Phytate changes were based upon differences between pH 5.8 and the pH levels tested. With RF, adjustment of pH to 3.5 and 5.15 increased phytic acid losses from 52% (seen with dispersion dialyzed without previous pH adjustment, i.e., at pH 5.8) to 85% and 88%, respectively, while adjusting pH to 9.0 did not greatly affect losses. Similarly with RPC, lowering the pH to 3.5 and 5.15 increased phytic acid losses from 31% (seen at p 5.8) to 65% and 55%, respectively, whereas pH adjustment to 9.0 resulted in a 26% loss of phytate. Evidently the nature of the phytate–protein complex is governed essentially by pH, and pH adjustment prior to dialysis disrupts those complexes. Generally, the phytate losses were greatest at acid pH values, i.e., at pH values that are below the isoelectric pH of most of the proteins present in rapeseed and that concurrently maintain optimal phytate solubility. With rapeseed, a large portion of the protein (20–40%) is characterized by an isoelectric pH close to 11 while the other proteins have isoelectric

points spread out in the interval from pH 4 to pH 8 (Gillberg and Tornell, 1976).

The overall greater losses observed with RF as compared to RPC may be attributed to the effect of heat treatment on the protein and, hence, on the strength of the protein–phytate interactions. The heat treatment involved in the production of RPC (Tape et al., 1970) probably resulted in an uncovering of the histidine groups, making the protein more basic (Gillberg and Tornell, 1976). This increased the strength of the electrostatic interaction with negatively charged phytic acid rendering the binary complex more stable and, hence, less easily disrupted. Therefore, heat treatment reduces the solubility of both phytic acid and nitrogen (Gillberg and Tornell, 1976).

While phytic acid losses were lowest at alkaline pH for both RF and RPC (50 and 26% respectively), these losses were improved to over 70% upon addition of EDTA (Figures 1 and 2). The effect of EDTA in enhancing phytate losses was more pronounced with RPC, however, as losses seen with pH adjustment to 9.0 are much less than those seen with RF. The success of EDTA in greatly enhancing phytate losses at pH 9.0 suggests the occurrence of a ternary protein–mineral–phytate complex at alkaline pH, the integrity of which depends on the presence of multivalent cations such as Ca^{2+}. The preferential binding of these cations to EDTA rather than phytate (Cheryan, 1980), with the formation of soluble cation–EDTA complexes, prevented the formation of a protein–mineral–phytate complex at alkaline pH. The Ca–EDTA complex can then be dialyzed out.

Failure of EDTA to remove all of the phytate present indicates that, even at pH 9.0, some binary protein–phytate complex may be present. Because the isoelectric pH of 20–40% of the proteins is close to 11, those proteins would still possess a positive charge at pH 9.0 and so could, theoretically, form a binary complex with the negatively charged phytic acid. In addition, the fact that the phytate losses seen with both RPC and RF upon addition of EDTA are comparable supports the notion that heat affects the binary protein–phytate interaction. As this interaction should remain unaffected by EDTA treatments, phytic acid losses occurring would be similar for both RF and RPC.

At pH 3.5, the addition of CaCl₂ not only failed to improve phytate losses but also reduced losses. This may be attributed to a couple of factors. First, in a dialysis system such as the one used here, where dialysis was carried out for 7 days against distilled water, change in the pH of the retentates might be expected. Examination of the retentates showed that after 1 day of dialysis, the pH rose from 3.5 to 4.5 and by the second day, the pH reached 5.0. This rapid rise in pH affected the charge of the protein, as well as the solubility of the Ca salts of phytate. Thus, the presence of excess Ca may serve to enhance the formation of the ternary protein–mineral–phytate complex. This formation would have been unlikely to occur had the pH been maintained at pH 3.5. The reduced loss of phytate observed with the addition of high concentration of CaCl₂ may also be due to the complex solubility behavior of mixed salts of phytic acid. An interaction is believed to occur between Zn and Ca such that if the Zn:Ca ratio is very wide, e.g., 0.00025 M Zn:0.03 M Ca, then Ca enhances the incorporation of Zn into phytate, i.e., there is an increased insolubility of Zn phytates in the presence of high concentrations of Ca (Byrd and Matrone, 1965). The Zn contents of RF and RPC are 75 and 120 ppm, respectively, while the CaCl₂ concentrations added ranged from 0.25 to 0.75 M; thus, the great excess of Ca as compared to Zn can
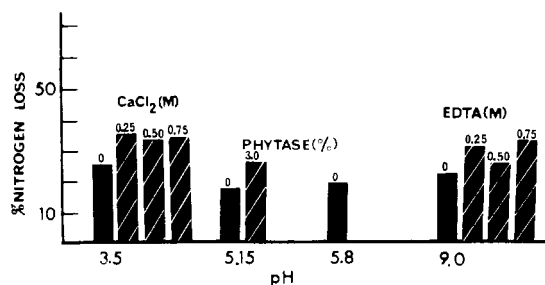
40   J. Agric. Food Chem., Vol. 32, No. 1, 1984

Serraino and Thompson

Figure 3. Effect of pH adjustment, EDTA, CaCl₂, and phytase enzyme treatment on nitrogen losses in rapeseed flour.

Figure 4. Effect of pH adjustment, EDTA, CaCl₂, and phytase enzyme treatment on nitrogen losses in rapeseed protein concentrate.

be appreciated. Presumably, with high Ca concentrations, Ca^{2+} increases the cationic environment sufficiently to initiate coprecipitation with Zn to form insoluble CaZn-phytate (Cheryan, 1980).

The phytase enzyme treatment proved to be totally effective in removing the phytic acid in both RF and RPC (Figures 1 and 2). Phytase, or myo-inositol-hexaphosphate phosphohydrolase, hydrolyzes phytic acid to myo-inositol and inorganic phosphate (Florkin and Stotz, 1964). It is the lack of this endogenous enzyme system that renders phytic acid generally unavailable to humans and monogastric animals (Cheryan, 1980). It appears that this enzyme treatment was an effective means of lowering phytate content in food systems.

Nitrogen Losses. Different methods were tested for phytic acid removal from RF and RPC to render the protein free of this compound and hence more acceptable for human and animal nutrition. Thus, it is important that the nitrogen losses with the methods and treatments applied are minimal. Since a dialysis membrane with a molecular weight cutoff of 6000–8000 was used and since the low molecular weight proteins of rapeseed are characterized by a molecular weight of about 13 000 (Gillberg and Tornell, 1976), the protein losses through the membrane should be minimal and restricted to small protein subunits or peptides. Most of the nitrogen losses occurring are most likely nonprotein nitrogen, which constitutes approximately 28% of total nitrogen in rapeseed (Miller et al., 1962; Bhatty, 1973). In fact, Figures 3 and 4, which show nitrogen losses upon dialysis of RF and RPC, respectively, illustrate that this value was not greatly exceeded. The nitrogen losses in RPC were relatively lower than for RF. This may be attributed to differences between the nonprotein contents of RF and RPC as a result of the treatment involved in the production of the latter (Tape et al., 1970).

Mere pH adjustment did not greatly affect nitrogen losses, which ranged from 18% to 23% for RF and 9% to 10% for RPC over the pH ranges tested, i.e., 3.5–9.0. Treatment with CaCl₂ and EDTA enhanced the losses to values exceeding 30% for RF and 17% for RPC while nitrogen losses observed with the phytase treatment were only 25 and 11% for RF and RPC, respectively. Ca salts have been shown to influence the solubility of nitrogen; at pH 4–6, the presence of CaCl₂ increased the solubility of nitrogen in rapeseed isolate (Gillberg, 1978). The addition of salt may affect the conformation of polyelectrolytes such as proteins, as well as the charge of the proteins, and, thus, may result in the dissociation of existing com-
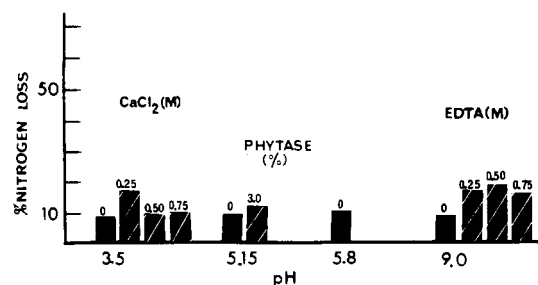
plexes and in the formation of new ones (Gillberg, 1978). This dissociation may result in the release of soluble low molecular weight proteins or polypeptides. Similarly, disruption of protein–phytate complexes with the release of soluble low molecular weight polypeptides can serve to explain any protein nitrogen losses resulting from EDTA and phytase treatments.

In summary, it is evident that pH is a major determinant of the nature and degree of interaction that occurs between phytic acid and protein in rapeseed. EDTA was successful in removing phytate from both RF and RPC at pH 9.0, indicating the presence of a ternary protein–mineral–phytic acid complex at alkaline pH. Binary complexes are believed to occur at pH 4, that is, below the isoelectric point of rapeseed proteins, and at alkaline pH values below pH 11, which is the isoelectric pH of the basic amino acids. Phytase enzyme treatment is undoubtedly a successful method for phytate removal from both RF and RPC and merits consideration for practical application on a large-scale basis. While the dialysis method is certainly not practically applicable in industry, ultrafiltration techniques can probably be substituted for large-scale operations.

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