Costello et al. (4) have recently determined the 12 separate $pK_a$ values of phytic acid by means of $^{31}P$ NMR spectroscopy. These determinations were done with tetrabutylammonium cation. They reported $pK_a$ values ranging from 1.1 to 12. The differences between their data and those of the present investigation can be attributed to their use of the tetrabutylammonium cation. Thus, it is to be expected that the large tetrabutylammonium cation would bind to the phytate anion more weakly than a cation such as $K^+$. This weaker binding would undoubtedly be reflected in the $pK_a$ values.

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# Preparation of Low-Phytate Rapeseed Protein by Ultrafiltration: I. The Aqueous Extraction of Phytate from Deoiled Rapeseed Meals

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## ABSTRACT

The extraction of phytate and nitrogen- and phosphorus-containing compounds from Tower and Candle variety rapeseed meal and flour using aq sodium chloride solution has been investigated. Single-stage extraction experiments were made varying the v/n ratio from 10 to 500 mL of extract solution per g of sample and using an extract solution containing 0-10% (w/v) sodium chloride. The pH of the extract solution was varied from 3.0 to 10.0. It was found that phytate and phosphorus-containing compounds can be completely extracted whereas the extent of extraction of nitrogen-containing compounds ranged from 27 to 75% and depended on the extent of protein denaturation of the sample. The above observations were found to be independent of the pH of the extract solution. The extraction method is viewed as a first step in the membrane preparation of a low-phytate rapeseed protein.


## INTRODUCTION

Rapeseed is an important source of vegetable oil. Associated with the production of rapeseed oil is rapeseed meal. Current interest in developing the meal as a new source of food quality protein is a direct consequence of the fact that it has a well-balanced amino acid composition. However, there are antinutrients associated with the edible protein, i.e., glucosinolates, erucic acid and phytates. Glucosinolates and erucic acid are the antinutritional factors which are themselves physiologically deleterious. Phytates are the antinutritional factors which induce a physiological deficiency, i.e., they affect the availability of minerals in the diet.

The methods proposed for inactivation or removal of glucosinolates from rapeseed proteins are numerous. Most detoxification methods consist of variations of heat inactivation and/or aqueous extraction. The most important development has been the breeding of low glucosinolate-low erucic acid varieties, e.g., Tower variety. However, using detoxified rapeseed protein (i.e., free from glucosinolates) Eklund (1) has shown that phytate has a detrimental effect on zinc metabolism. This conclusion has been reconfirmed (2,3).

Rapeseeds, like other oilseeds, are particularly rich in phytates. Nutritionally, phytate binds strongly with several essential minerals to form insoluble complexes. It is therefore capable of inducing a wide variety of physiological deficiencies, depending on the first limiting element in a specified diet.

The structure and configuration of phytic acid is shown in Figure 1. Phytate, the metallic salt of phytic acid, was first encountered as early as 1872 (4); however, its structure was not established until recently (5). This perhaps explains why no direct analytical method for phytate is available to date, and why so few studies have been published on its separation from plant protein.

The objectives of this project are (a) to determine the most favorable condition for aqueous extraction of phytate from deoiled rapeseed meal, and (b) to develop a suitable porous membrane process to effectively remove the extracted phytate from the dissolved proteins prior to their recovery as protein isolates. This paper deals with the aqueous extraction of phytate from deoiled rapeseed meals, a first step toward the preparation of low-phytate rapeseed protein by membrane separation.

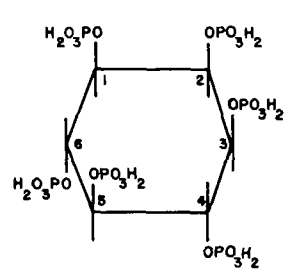


**FIG. 1. Structure and configuration of phytic acid, myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate).**

## MATERIALS AND METHODS

The 3 rapeseed samples used were deoiled commercial Tower meal with hull, dehulled-deoiled Tower meal, and Candle flour. The deoiled commercial Tower meal FRI-75-1 and Candle flour FRI-77-3/4/4 (1024) were obtained from the Food Research Institute of Agriculture Canada. The dehulled-deoiled Tower meal was obtained from our chemical engineering department and was prepared by the procedure previously described (6). Table I is an analysis of the nitrogen, total phosphorus and phytate phosphorus contents of the samples on an air-dried basis.

TABLE I

**An Analysis of the Total Nitrogen, Total Phosphorus and Phytate Phosphorus Contents of the Rapeseed Samples**

| Type of samples | Weight % of air-dried, deoiled meals | | |
|---|---|---|---|
| | % Total Nitrogen | % Total phosphorus | % Phytate phosphorus |
| Commercial Tower meal | 6.68 | 1.13 | 0.84 |
| Candle flour | 6.66 | 1.40 | 1.16 |
| Tower meal | 7.53 | 2.03 | 1.54 |

The extraction experiments were done in a mechanical shaker at 24 ± 1 C. The pH of the extract solutions were adjusted with reagent grade hydrochloric acid and sodium hydroxide when applicable. The extraction procedure was as follows. A weighed amount of rapeseed meal or flour and 100 mL of prepared extraction solution and glass beads were placed in a stoppered 200-mL Erlenmeyer flask and mechanically shaken for 3 hr. The insoluble material was removed by centrifuging at 2,500 rpm for 10 min followed by filtering through ashless filter paper of medium retentivity. The supernatant liquid and insoluble solids (when applicable) were analyzed for nitrogen, total phosphorus and phytate phosphorus content. The determination of Kjeldahl nitrogen content was according to Lang (7) whereas the total phosphorus and phytate phosphorus were determined according to Pons et al. (8) with modifications by the authors. Dissolved solute was calculated as: (concentration of solute in the supernatant liquid) × (initial volume of extraction solution). Most analyses were done in duplicate and precision obtained was within 2%.

## RESULTS AND DISCUSSION

Preliminary extraction tests done in the laboratory using various acid, alkaline and salt solutions at different concentrations and pH indicated that sodium chloride solution was the best dispersing agent for phytate extraction.

The effect of sodium chloride concentration on dissolution of substances containing nitrogen and phytate gave similar types of curves for all 3 rapeseed samples. In Figure 2, a >90% dissolution of phytate was obtained for all 3 samples with a 4% (w/v) sodium chloride solution. The extent of dissolution for nitrogen-containing substances was quite different between samples: 27% for commercial Tower meal, 40% for Tower meal and 75% for Candle flour. Lower nitrogen solubility indicates a higher extent of protein denaturation. The results were not surprising because the samples underwent different degrees of heat treatment during preparation. Heat treatment received by the commercial Tower meal for enzyme inactivation was the most severe. On the other extreme, Candle flour was specially prepared to minimize protein denaturation. Figure 2 also indicates the limited water solubility of phytate and nitrogen compounds of the samples and the dramatic increase in solubility when salt is added as a dispersing agent.

The effect of v/n ratio on dissolution of nitrogen and phosphorus compounds from the samples is shown in Figure 3. The results indicate nitrogen compounds dissolve more readily than phosphorus compounds, and a v/n ratio greater than 200 mL/g is required to dissolve all phosphorus and soluble nitrogen compounds. Selective dissolution is possible at v/n ratios less than 200 mL/g. As phytate

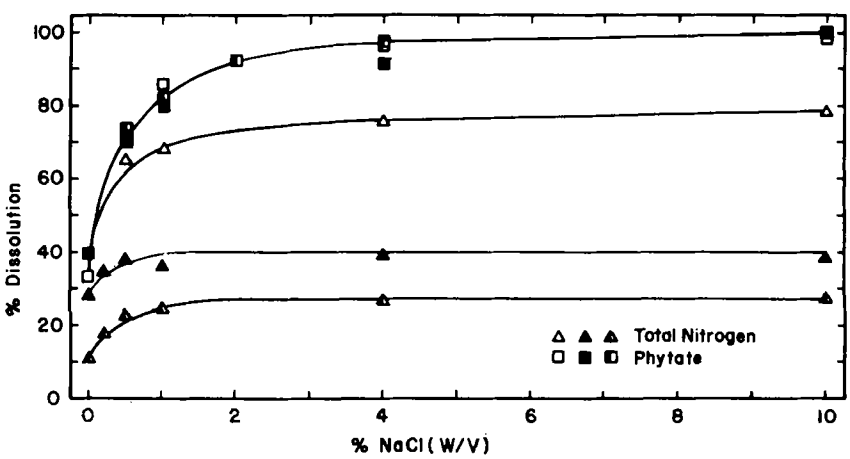


FIG. 2. Effect of NaCl concentration on the dissolution of phytate- and nitrogen-containing substances (pH = 6.3, v/n = 200 mL/g). △ □ Candle flour; ▲ ■ Tower meal; ◭ ◧ commercial Tower meal.

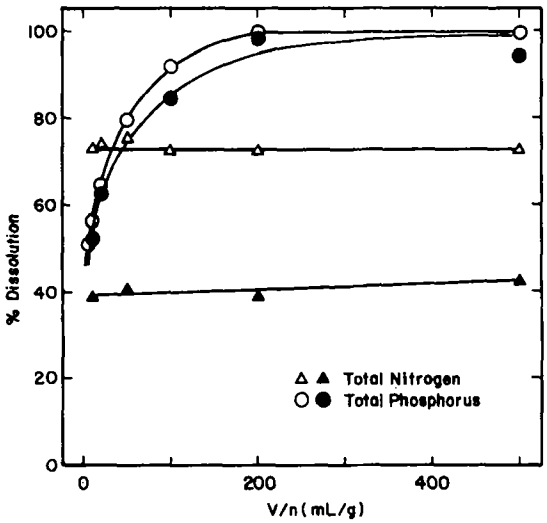


FIG. 3. Effect of v/n ratio on the dissolution of nitrogen- and phosphorus-containing substances (pH = 6.3, 4 [w/v] % NaCl solution). ▲ ● Tower meal; △ ○ Candle flour.

phosphorus accounted for more than 75% of the total phosphorus, solubility data obtained for total phosphorus can be used as a good approximation for phytate.

The influence of solution pH on rapeseed extraction was investigated using sodium chloride levels of 0.0, 0.5 and 1.0% (w/v) and v/n ratios of 50 and 20 mL/g. Within the tolerable pH of extraction, i.e., between 3.0 and 10.0, solution pH showed no significant influence on dissolution of nitrogen- and phosphorus-containing substances under all test conditions. The results are presented in Tables II and III. Gillberg and Törnell (9) reported that dissolution curves of nitrogen- and phosphorus-containing substances vary in a complicated manner with pH when rapeseed is extracted with deionized water. However, they used a different variety of rapeseed and employed a different extraction procedure. Their extraction was carried out for 30 min at constant pH and did not represent equilibrium data. This may be sufficient to account for the differences found between our work and theirs. In Table II, only at extremely high and low pH values did the solution pH affect the dissolution as shown by the commercial rapeseed sample. The complex variation of phytate solubility at extreme

TABLE II

**Effect of pH on the Dissolution of Nitrogen- and Phosphorus-Containing Substances Using 1% (w/v) NaCl Solution and v/n = 50 mL/g (% Dissolution)**

| | Commercial Tower meal | | | Tower meal | | | Candle flour | | |
|---|---|---|---|---|---|---|---|---|---|
| pH | Total N | Total P | Phytate P | Total N | Total P | Phytate P | Total N | Total P | Phytate P |
| 0.57 | 30.87 | 97.73 | 100.00 | – | – | – | – | – | – |
| 1.04 | 25.72 | 79.12 | 87.12 | – | – | – | – | – | – |
| 1.43 | 24.41 | 61.49 | 64.81 | – | – | – | – | – | – |
| 2.03 | 20.31 | 71.43 | 79.84 | – | – | – | – | – | – |
| 2.73 | 27.54 | 87.67 | 100.00 | – | – | – | – | – | – |
| 3.44 | 30.44 | 66.30 | 79.83 | – | – | – | – | – | – |
| 3.52 | – | – | – | 37.73 | 70.69 | 78.68 | 72.63 | 77.61 | 61.25 |
| 4.00 | – | – | – | 38.47 | 70.12 | 72.59 | 69.92 | 74.12 | 63.07 |
| 4.46 | 32.32 | 64.38 | 77.32 | – | – | – | – | – | – |
| 5.05 | – | – | – | 37.90 | 67.42 | 69.17 | 72.08 | 73.01 | 59.99 |
| 5.34 | 30.52 | – | – | – | – | – | – | – | – |
| 5.63 | 35.43 | 67.41 | 77.12 | – | – | – | – | – | – |
| 6.33 | – | – | – | 39.92 | 68.50 | 74.80 | 70.63 | 75.07 | 58.48 |
| 6.38 | 28.11 | 62.40 | – | – | – | – | – | – | – |
| 6.53 | 27.19 | 61.02 | – | – | – | – | – | – | – |
| 6.68 | 28.21 | 63.01 | 69.01 | – | – | – | – | – | – |
| 8.35 | 31.97 | 62.60 | 69.17 | – | – | – | – | – | – |
| 9.46 | – | – | – | 36.28 | 65.90 | 70.93 | 70.98 | 74.94 | 61.76 |
| 9.83 | 29.87 | 60.44 | 65.14 | – | – | – | – | – | – |
| 10.53 | 30.10 | 54.10 | 58.48 | – | – | – | – | – | – |
| 11.20 | 27.92 | 31.31 | 12.34 | – | – | – | – | – | – |
| 11.74 | 32.09 | – | – | – | – | – | – | – | – |
| 11.93 | 30.21 | 14.98 | 13.34 | – | – | – | – | – | – |
| 12.82 | 55.78 | 55.14 | 47.44 | – | – | – | – | – | – |
| 13.86 | 97.80 | 100.00 | 100.00 | – | – | – | – | – | – |

TABLE III

**Effect of pH on the Dissolution of Nitrogen- and Phosphorus-Containing Substances in Tower Meal Using Distilled Water and 0.5% (w/v) NaCl Solution**

| | % Dissolution | | | v/n = 20 mL/g | | |
|---|---|---|---|---|---|---|
| | Distilled water | | | 0.5% w/v NaCl solution | | |
| pH | Total N | Total P | Phytate P | Total N | Total P | Phytate P |
| 3.3 | – | – | – | 40.93 | 56.61 | 55.23 |
| 3.4 | 34.79 | 48.90 | 43.13 | – | – | – |
| 4.0 | 33.34 | 47.54 | 44.50 | 39.67 | 54.92 | 53.33 |
| 4.5 | 34.39 | 47.17 | 43.50 | – | – | – |
| 4.9 | – | – | – | 39.53 | 51.36 | 52.19 |
| 5.4 | – | – | – | 39.64 | 53.35 | 52.42 |
| 5.5 | 32.93 | 47.18 | 42.30 | – | – | – |
| 6.1 | – | – | – | 43.28 | 55.40 | 52.46 |
| 7.0 | 33.21 | 47.43 | 41.72 | – | – | – |
| 9.9 | – | – | – | 38.60 | 52.68 | 51.24 |
| 10.0 | 34.50 | 46.58 | 41.97 | – | – | – |

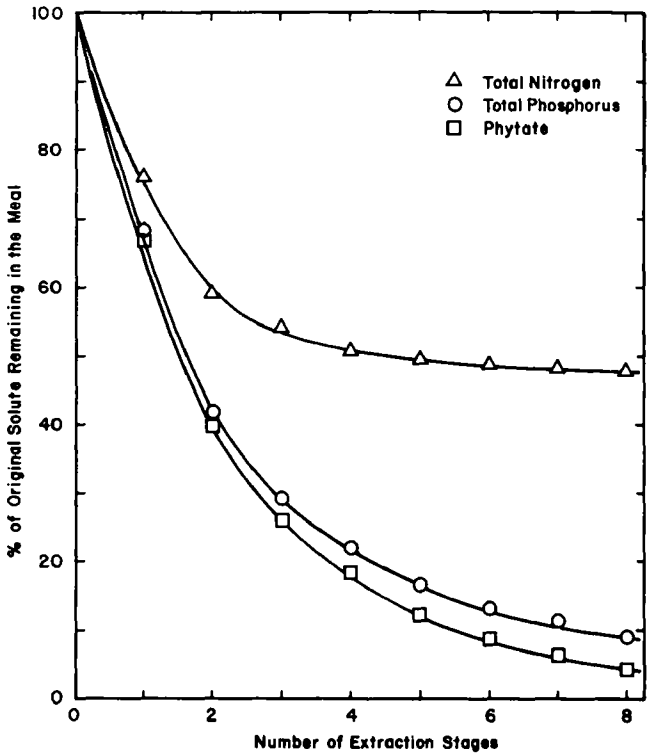


**FIG. 4. Multistage cross-current extraction of Tower meal (pH = 6.3, v/n = 1.0 mL/g, 4 [w/v] % NaCl solution).**

pH can be qualitatively explained by considering that phytic acid can react with metal ions as well as protein during extraction. This explanation was given by Gillberg et al. However, extreme pH values were outside the interest of our extraction criterion, i.e., to minimize protein denaturation.

The use of a single extraction stage has limitations with regard to the design of a processing sequence. If it is only desired to produce a low-phytate residue from a deoiled meal such as the commercial meal used in this study, a single extraction using a 4% (w/v) sodium chloride solution and a v/n ratio greater than 200 mL/g would suffice. However, if it were desired to minimize the volume of extraction liquid, multistage extraction would have to be used. This would be so if the extraction liquid were to be processed to recover a low-phytate protein isolate. The results of a multistage cross-current extraction of Tower meal using fresh extract solution at a v/n ratio of 10 mL/g/stage is shown in Figure 4. This shows that 95% of the phytate phosphoruc and virtually all of the soluble nitrogen compounds can be extracted with 8 stages, producing an overall v/n ratio of 80 mL/g.

This investigation indicates that phytate can be extracted from deoiled rapeseed meal and flour using dilute sodium chloride solutions. The solubility of phytate phosphorus appears to be independent of the solubility of nitrogen compounds, and a high degree of removal can be achieved using standard extraction techniques.

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