# The Production of Chinese Rapeseed Protein Isolates by Membrane Processing

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**A membrane-based process was developed for the production of Chinese rapeseed protein isolates, which consisted of alkaline extraction of protein at pH 12.0 from $CH_2OH/NH_3/H_2O$-hexane-extracted Chinese rapeseed meal, isoelectric precipitation at pH 6.5, ultrafiltration followed by diafiltration to concentrate the remaining soluble protein, and freeze-drying. No chemical treatment was required for the removal of phytates from the protein. Three products were obtained with high protein recovery: precipitated and soluble protein isolates, each containing 90 to 100% protein (N% × 6.25), and a meal residue with about 25% protein, suitable for animal feed. The two protein isolates were low in phytates, light in color and bland in taste. Chinese rapeseed protein was easier and more economical to treat than canola protein due to the shift of pH for isoelectric precipitation and the elimination of the need for further phytate reduction.**

KEY WORDS: Alkaline extraction, Chinese rapeseed, diafiltration, isoelectric precipitation, phytic acid, protein isolates, ultrafiltration.

As a result of increased utilization of rapeseed oil for cooking and for manufacture of margarine, shortening and salad oil, rapeseed is now ranked third among all vegetable oilseeds in total tonnage. Annual rapeseed production exceeds twenty million tons, with China as the largest producer and Canada as the largest exporter. The rapeseed cultivars now grown in Canada, known as "canola," have been genetically improved to contain low levels of erucic acid (<2% in the oil) and glucosinolates (<30 μM/g in the meal). In China, however, conventional varieties, high in both erucic acid and glucosinolates, are still grown.

The seed contains about 40% oil and 17 to 26% protein, yielding a meal containing up to 50% protein after oil extraction. Rapeseed meal protein has a well-balanced amino acid composition (1); it may thus be considered a potential source of food-grade protein. Unfortunately, the presence of undesirable compounds, such as glucosinolates, phytates, phenolics and fiber, prevents use of the meal in human food, and thus, most of the meal is used in animal feed or only as an organic fertilizer.

Over the past twenty years, a large variety of processes have been developed aimed at producing protein materials that are suitable for human food from rapeseed. None of these techniques, however, has been commercialized due to low product recovery, poor quality or high cost. A unique rapeseed processing scheme was developed in our laboratories, which involved the combined use of two immiscible organic solvents, methanol containing ammonia and hexane (2,3). This two-phase solvent extraction method produced a high-quality rapeseed meal and an edible oil from the seed. The process was then extended by the development of a membranne-based process for rapeseed protein isolation, comprising five main steps: alkaline extraction, calcium chloride treatment, isoelectric precipitation, ultrafiltration and diafiltration, and drying (4). The two-phase solvent extraction system, with a few modifications, was also successful for Chinese rapeseed (5). Although Chinese rapeseed contains much more glucosinolates than canola seed, the process was readily adapted to produce a high-quality meal for feed use with a high protein recovery.

Therefore, the objective of this project was to adapt the membrane-based protein isolation process to two-phase solvent-extracted Chinese rapeseed meal, thus producing high-quality protein isolates that represent a potential source of protein for human consumption.

## MATERIALS AND METHODS

*Preparation of starting meal.* The rapeseed used in this project was a typical Chinese variety, Nin-U 7, which was obtained from the Nanjing Institute of Chemical Technology (Nanjing, People's Republic of China).

Chinese rapeseed meal was produced by the modified two-phase solvent-extraction method (5) on a bench scale. For the preparation of each batch, 90 g of seed was ground in a commercial Osterizer blender at the highest speed for 2 min and was then mixed with 600 mL methanol/ammonia/water solution for 2 min at the same speed. After setting for 15 min, 600 mL hexane was added, and the mixture was blended for another 2 min. The meal was recovered by vacuum filtration through Whatman No. 42 filter paper, washed twice with 150 mL methanol/ammonia/water solution each time, twice with 150 mL methanol, and dried in a well-ventilated place overnight at room temperature. The residual oil in the meal was extracted with hexane in a Soxhlet extractor for 12 h, and the meal was dried.

Seventeen batches resulted in 770 g, with the following composition: moisture, 7.23 ± 0.12%; protein, 54.8 ± 0.5% (on moisture-free basis); and phytic acid, 3.98 ± 0.07% (on moisture-free basis).

*Alkaline extraction.* Twenty grams of meal (on moisture-free basis) was dispersed in 360 g distilled water at room temperature for 30 min (one hour of extraction did not increase the protein solubility). The pH of the solution was adjusted and maintained at the target value with NaOH. A pH range from 10.5 to 12.5 was examined in increments of 0.5.

The slurry was centrifuged in a Sorvall RC-5B centrifuge (Ivan Sorvall, Inc., Norwalk, CT) at 5,000 rpm for 15 min. The supernatant was filtered through Whatman No. 41 filter paper. Aliquots were taken from the filtrate to determine the dissolved nitrogen concentration. The meal residue was washed twice with 120 mL aqueous alkali of the same pH as the extraction solution and oven-dried at 105°C overnight.

*Isoelectric precipitation.* The precipitation of dissolved protein at different pH values was examined. Meal was extracted with NaOH at pH 12.0 and a solvent-to-meal ratio of 18 as described above. The extract and all washing liquids were combined. Samples (400 mL) were acidified with HCl to pH values ranging from 3.0 to 9.0 in increments of 0.5. After centrifugation at 5000 rpm for 20 min, the precipitates were washed with water acidified to the precipitation pH, centrifuged again and oven-dried at 105°C overnight.

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*Preparation of protein isolates.* The process of Tzeng *et al.* (4) was followed. It consists of five major steps: alkaline extraction, isoelectric precipitation, ultrafiltration, diafiltration and drying (Scheme 1). As $CaCl_2$ facilitates the removal of phytic acid from protein (6), a control run was conducted in which 15% $CaCl_2$ (by weight of starting meal) was added to the extract prior to isoelectric precipitation.

Meal was extracted at pH 12.0 as described above. The extract was combined with all the washings and acidified with HCl to pH 6.5. The pH was maintained for 15 min for protein aggregation, and the suspension was centrifuged at 5000 rpm for 20 min. The supernatant was decanted and polish-filtered by using Whatman No. 41 filter paper. The precipitate was washed with five times its weight of distilled water on a wet basis and centrifuged again.

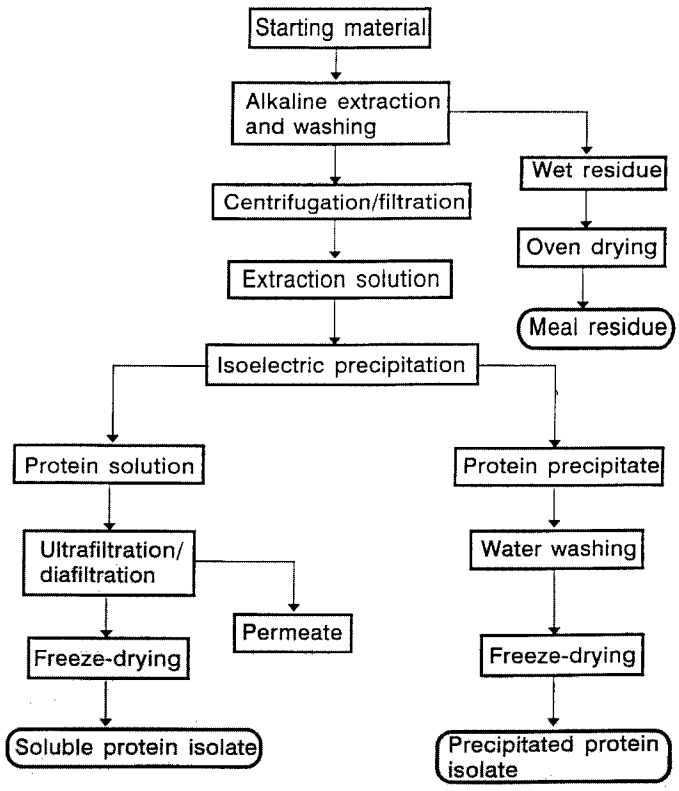
The supernatant and the washing liquid were combined and subsequently ultrafiltered at a concentration factor of 10. Immediately after ultrafiltration, diafiltration was carried out at a diavolume of 5, in which distilled water was used as fresh solvent to replace the permeate removed.

Both the washed precipitate and the retentate from membrane processing were freeze-dried to give two protein isolates.

*Membrane processing unit.* An Amicon hollow-fiber concentrator, Model CH4 (Amicon Corp., Lexington, MA), was used in both ultrafiltration and diafiltration modes. The solution was pumped through a prefilter to remove fine suspended solids and then through an Amicon DIAFLO H1P10-20 hollow-fiber membrane cartridge. The membrane had a nominal molecular weight cut-off of 10,000 and a membrane area of approximately 0.05 $m^2$.

*Chemical analyses.* The moisture content was determined gravimetrically according to American Association of Cereal Chemists (AACC) Method 44-15A (7). Crude protein (N × 6.25) was determined by the Kjeldahl method, AACC Method 46-12 (7).

Phytate was determined according to Naczk *et al.* (8). Phytate phosphorus was determined colorimetrically according to the American Oil Chemists' Society Official Method Ca 12-55 (9). Phytic acid was calculated from phytate phosphorus by using a conversion factor of 3.55.




SCHEME 1

## RESULTS AND DISCUSSION

*Protein and phytic acid extractability.* The nitrogen extractability curve of rapeseed meal has a rather complex shape. According to Gillberg and Tornell (10), in NaOH extract, the curve exhibited two broad minima at pH 3.5–4.8 and 7.0–8.0, and at these pH values, 35 and 60% of the protein are extracted, respectively. This could be attributed to the fact that rapeseed has a complicated protein composition and contains proteins with isoelectric points in the pH range 4–11 and molecular weights from 13,000 to 320,000 D (11). However, high pH (>11) considerably increases the nitrogen extractability from rapeseed meal (4,10).

Chinese rapeseed is a different variety from canola seed in many aspects. The influence of pH on its nitrogen and phytic acid extractability are shown in Figure 1. As expected, the nitrogen extractability of Chinese rapeseed meal increased as the extraction pH went up. There was a marked rise in nitrogen extractability (from 21.6 to 55.1%) between pH 10.5 and 11.0, which was not observed with canola meal. In the range from pH 11.0 to 12.0, the nitrogen extractability rose steadily with increasing pH, and because no significant further increase was found between pH 12.0 and 12.5, higher values were not investigated. At pH 12.0, over 70% of the meal nitrogen was dissolved, which was higher than the 55 to 65% obtained with two-phase solvent-extracted canola meal (4,6) but was still lower than the nitrogen extractability of hexane-defatted canola meal, which was up to 90% at pH 12.0 (4). Naczk *et al.* (12) suggested that the decrease in protein

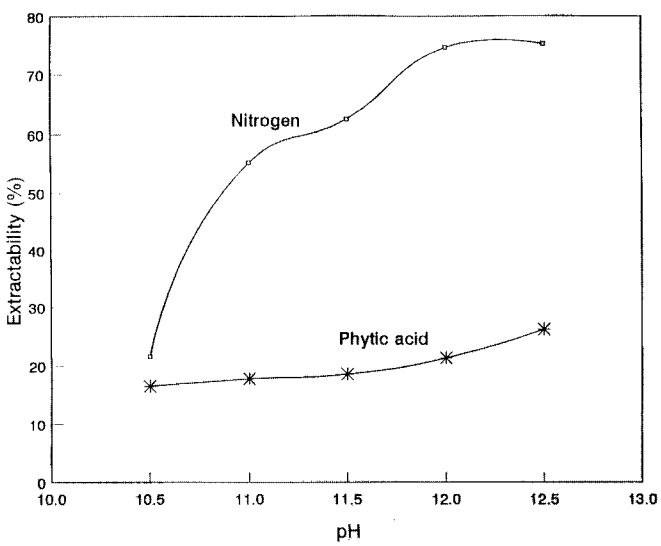



**FIG. 1. Effect of pH on nitrogen and phytic acid extractability of Chinese rapeseed meal.**

extractability of methanol/ammonia/water-hexane-extracted rapeseed meal was due to the formation of insoluble protein aggregates by methanol dehydration. The phytic acid extractability of our meal was low in the pH range 10.5-12.5. At pH 12.0, only 21.3% of the phytates in the meal was dissolved in the extract, which was still slightly higher than that of canola meal. In the work by Diosady *et al.* (6), the phytic acid extractability of methanol/ammonia/water-hexane-extracted Westar canola meal (Canola Council of Canada, Winnipeg, Manitoba, Canada) was 18.1% in aqueous NaOH at pH 12.0. Earlier, Tzeng *et al.* (4) reported an extractability of 13.5% for altex canola meal at pH 12.0 and a minimum extractability of nearly 0% at pH 11.0, which closely agreed with the result obtained by Gillberg and Tornell (10). Chinese rapeseed meal did not show a minimum at pH 11.0, implying that the phytic acid extractability of rapeseed meal depends, to a certain extent, on the variety.

The low phytic acid extractability is mainly caused by a shift in the equilibria of the ternary complexes of rapeseed proteins, cations and phytic acid due to the presence of excess sodium ion (13). At intermediate pH (7-11), phytic acid reacts with metal ions and proteins to form a ternary phytic acid-cation-protein complex, with increased stability with increasing pH up to 10 (13). Accordingly, protein and phytic acid extractability increases with pH. As pH goes higher than 10, the complex becomes much less stable, and the phytic acid solubility decreases drastically around pH 10. Gillberg and Tornell (10) explained this phenomenon on the grounds that phytic acid is probably attached to the basic groups of lysine or arginine residues in the protein molecules because they are the only amino acid residues to be positively charged at pH 10. The $pK_a$ values of these two residues are 10.53 and 12.48, respectively. This indicates that, from pH 10 to 12.5, the number of positive charges of the protein molecules will decrease greatly, and the stability of any protein-phytic acid complexes will thus be substantially reduced.

Therefore, in preparing Chinese rapeseed protein isolates, it is still advisable to extract meal proteins at a high pH, e.g., pH 12, although Chinese rapeseed meal showed a slightly greater phytic acid extractability than canola meal. It was reported that the alkaline treatment of proteinaceous materials could produce lysinolalanine, which may be toxic to humans. Deng *et al.* (14) clearly demonstrated the need for careful control of the conditions during protein extraction, to prevent the formation of potentially harmful levels of lysinoalanine, and indicated that rapeseed protein products prepared by the processes developed in our laboratories do not represent a significant hazard due to the formation of lysinoalanine content. However, long contact at high pH (>12) is not recommended for alkaline extraction of rapeseed protein.

*Precipitability of Chinese rapeseed protein.* It was previously shown in our laboratories that canola protein has the lowest solubility near pH 3.5, where some 50% of the dissolved protein was precipitated (4,6). Because the detailed protein composition is specific to each variety, several different isoelectric points were used by previous researchers for precipitation (10,15). Chinese rapeseed is different from canola in many aspects, and therefore, the effect of pH on the precipitation of extracted Chinese rapeseed protein was investigated to find the optimal conditions for the recovery of protein dissolved in NaOH solution. The experimental results demonstrated the distinct differences of the precipitation behavior of Chinese rapeseed protein from that of canola protein.

As shown in Figure 2, at low pH (<4), only a small fraction of the dissolved Chinese rapeseed protein was precipitated, and as the pH rose, the protein recovery increased strikingly. It is quite obvious that pH 3.5 is not appropriate for precipitating Chinese rapeseed protein because of the low yield attained (≈19%) at this pH. In the pH range from 4 to 5.5, both the dry matter amount and the protein recovery continued to increase but at a lower rate. The curve showed a broad precipitation maximum, covering a wide pH range from 5.5 to 8.0, within which over 50% of the extracted protein was recovered in the precipitate. As shown in Figure 2, between pH 5.5 and 8.0, the protein recovery-vs.-pH curve appears basically level, although there is an indistinct maximum of 54.9% at pH 6.5. Above pH 8.0, the protein recovery started to decrease. The maximum protein yield of 54.9% was similar to that reported by Girault (15). However, in comparison with the results previously obtained in our laboratories with canola meal, the protein recovery from precipitation of Chinese rapeseed protein was high. Tzeng *et al.* (4) attained a recovery of 50.1% of the dissolved nitrogen at pH 3.5 with hexane-defatted canola meal. With the $CH_3OH/NH_3/H_2O$-hexane-extracted canola meal, the protein recovery was only 47.1%, equivalent to 25.8% of the total meal protein.

The results indicate that, unlike canola protein, Chinese rapeseed protein has isoelectric points in the neutral pH range. Accordingly, in all subsequent experiments, pH 6.5 was used for isoelectric precipitation of Chinese rapeseed protein.

*Protein recovery.* Most of the nitrogen (<85%) in the starting meal was recovered as three usable products, including two high-quality protein isolates and the meal residue (Tables 1 and 2). Less than 15% of the total nitrogen was lost in either the permeate or transfers. In all the runs without $CaCl_2$ treatment, the precipitated protein isolate (PPI) had a protein content (N% × 6.25) of nearly 100% (Table 1), while the soluble protein isolate (SPI) contained approximately 90% protein. In contrast, with

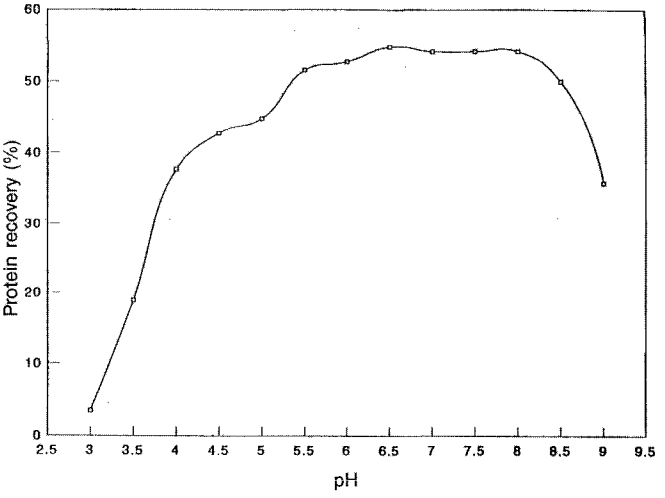



**FIG. 2. Effect of pH on protein recovery by isoelectric precipitation of Chinese rapeseed protein from pH 12.0 aqueous NaOH extract.**

TABLE 1

**Comparison of Protein and Phytic Acid Contents of Chinese Rapeseed Protein Fractions with and without $CaCl_2$ Treatment**

| Product | $CaCl_2$[a] (%) | Protein[b] (%) | Phytic acid[b] (%) |
|---|---|---|---|
| Precipitated protein isolate | 0 | 99.9 ± 0.4 | 0.39 ± 0.11 |
| | 15 | 97.2 ± 0.3 | 0.1 |
| Soluble protein isolate | 0 | 91.2 ± 0.4 | 0.83 ± 0.07 |
| | 15 | 90.8 ± 0.8 | 0.32 ± 0.10 |
| Meal residue | 0 | 25.5 ± 1.0 | 5.45 ± 0.29 |
| | 15 | 25.2 ± 0.8 | 5.59 ± 0.24 |

[a]The values for 0% $CaCl_2$ are averages of four replicate runs. Only a single run was made with $CaCl_2$ treatment.
[b]On moisture-free basis.

canola protein, the soluble isolate had a higher protein content than the precipitated isolate [PPI 96.8% vs. SPI 100.1% (6)]. The third product, meal residue (MR), contained 25.5% protein and should be suitable for animal feed.

The total isolate yield was 34.1% (20.5 ± 13.6) of the starting meal mass (Table 2). The isolates accounted or 60.0% of the nitrogen, with PPI making up 37.4% and SPI 22.6% (Table 2). The mass recovery of the protein isolates in this case was higher than what was previously reported by our laboratories on canola protein preparation. Tzeng *et al.* (4) obtained an isolate yield of 32.5% with hexane-defatted canola meal as the starting material, and Diosady *et al.* (6) attained 29.6% with $CH_3OH/NH_3/H_2O$-hexane-extracted canola meal. The protein recovery of the isolates was also higher than the 47 and 58% achieved with $CH_3OH/NH_3/H_2O$-hexane-extracted canola meal (4,6).

Some of the nitrogen lost during the process was non-protein nitrogen, which eventually found its way into the permeate.

*Removal of phytic acid.* Phytates are present in rapeseed meal at levels as high as 7%. They are strong chelating agents and affect the utilization of most multivalent metal ions, especially zinc and iron, by strongly binding them and thus reducing their bioavailability. Therefore, before rapeseed protein can be considered as an ingredient in the human diet, it is desirable to reduce the phytate levels to <1%.

According to Cheryan (13), pH plays an important role in the formation of phytate-protein comlexes. At low pH (<5.5), phytic acid is associated with protein by a salt-labile linkage, due to strong electrostatic interaction, because the protein has a net positive charge at a pH below its isoelectric point while phytic acid is strongly negatively charged at all pH values. Therefore, the removal of phytates from a protein system at low pH depends on the ability to dissociate the protein-phytate complex. It was observed that the presence of calcium chloride interferes with the formation of the protein-phytate complex because calcium ion is able to compete with the protein for phytic acid (13). Diosady *et al.* (6) concluded that, at a $CaCl_2$ level of 15% by weight of starting meal, about 60.5% of the dissolved phytic acid was removed by membrane processing, resulting in a canola SPI with only 0.65% phytic acid.

With Chinese rapeseed protein, however, even when no $CaCl_2$ was added to the alkaline extract prior to isoelectric precipitation, the phytic acid content in both isolates was well below 1% (Table 1). This could be attributed to the shift of pH for isoelectric precipitation from 3.5 to 6.5. Because at an intermediate pH (>6) the protein as a whole was negatively charged, Cheryan (13) proposed that a ternary complex, consisting of protein and phytic acid bonded by a multivalent cation, was formed. Unlike the binary complex formed at low pH, the ternary complex could be dissociated by excess $Na^+$. In the preparation of Chinese rapeseed protein isolates, the protein in the starting meal was first extracted with NaOH solution at pH 12.0, so when pH was brought down to 6.5 to precipitate the extracted protein, no ternary complexes were produced in the presence of sodium ions. Thus, the PPI was low in phytates. The free phytates were finally removed by membrane processing, resulting in a low phytate content in the SPI.

The results indicate that the majority of the phytate remained in the meal residue (Table 2). Apart from the part that went into the permeate, the loss of phytic acid may be due to mass loss or, more likely, to the errors in the phytic acid analyses of the oven-dried meal residue.

Although $CaCl_2$ was effective in aiding the removal of phytic acid from Chinese rapeseed protein, it will be unnecessary for commercial Chinese rapeseed protein production. Obviously, the elimination of this step would make the process simpler and more economical.

TABLE 2

**Mass, Nitrogen and Phytic Acid Distributions Among Products**

| Product | $CaCl_2$[a] (%) | Mass[b] (%) | Nitrogen[b] (%) | Phytic acid[b] (%) |
|---|---|---|---|---|
| Precipitated protein isolate | 0 | 20.5 ± 0.2 | 37.4 ± 0.4 | 2.0 ± 0.3 |
| | 15 | 21.2 | 37.6 | 0.0 |
| Soluble protein isolate | 0 | 13.6 ± 0.3 | 22.6 ± 0.8 | 2.8 ± 0.3 |
| | 15 | 13.2 | 21.8 | 1.0 |
| Meal residue | 0 | 58.5 ± 0.8 | 27.2 ± 1.1 | 80.1 ± 0.3 |
| | 15 | 57.8 | 26.6 | 81.2 |
| Loss[c] | 0 | 7.5 ± 0.4 | 13.0 ± 0.4 | 15.1 ± 0.6 |
| | 15 | 7.8 | 14.0 | 18.8 |

[a]The values for 0% $CaCl_2$ are averages of four replicate runs. Only a single run was made with $CaCl_2$ treatment.
[b]All values reported as percentage of original quantity in the starting meal.
[c]Includes the permeate.

With the optimized process, 60% of the protein in the meal is recovered in the isolates. The PPI contained up to 100% protein (N × 6.25), and the SPI had a protein content of around 92%. Both isolates were low in phytic acid (<1%), light in color and bland in taste. The MR, containing about 25% protein, could be used in animal feed. Based on their chemical composition, the isolates are suitable for human consumption. Their usefulness will depend on their functional properties, which will be reported separately.

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