Oil-Free Protein Isolates from Full-Fat, Dehulled Mustard Flour by Microfiltration

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ABSTRACT: New microfiltration (MF)-based aqueous processes for the extraction of oil-free protein isolates from full-fat, dehulled mustard flour have been developed. The processes used hydrophilic MF membranes to separate oil and protein. The most successful processing sequence consisted of alkaline extraction of oil and protein from the flour at pH 11, centrifugation to remove undissolved solids, and an initial microfiltration step to separate oil and dissolved protein. This was followed by proteolytic enzyme treatment of the retentate and a second-stage MF step to recover further protein. The proteolytic enzyme treatment was designed to break aggregated proteins into smaller fractions to permit their passage through the MF membrane. The permeates from the two microfiltration steps were ultrafiltered to concentrate the protein and remove antinutritional compounds and highly fragmented peptides. With this process, 60% of the protein originally in the flour was separated from the oil phase. Approximately 40% of the protein present in the flour was recovered in the form of two oil-free protein isolates—a soluble protein isolate containing 91% protein and a precipitated protein isolate containing 100% protein. The process proves the potential of MF as an effective method of extracting oil-free protein isolates from oilseeds without the use of organic solvents.

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KEY WORDS: Aqueous processing, enzyme, fouling, microfiltration, mustard, oil–protein separation, protein isolates.

The production of protein isolates from rape, soybean, peanut, and other oilseeds has been the subject of much research over the last five decades. In spite of being an accepted food ingredient, mustard is yet to receive the same attention. Mustard seed (*Sinapis alba*) has a high protein content (28 to 36%) and a well-balanced amino acid composition (1). The characteristic sharp flavor of mustard is caused by glucosinolates and their reaction products. Previous work in our laboratory showed that membrane processing using ultrafiltration (UF) membranes can be used to reduce concentrations of glucosinolates and phenolics in canola/rapeseed and mustard meals to acceptable levels (2,3). Once free of flavor components, protein isolates from mustard may be used as emulsifiers, water-binding agents, and for texture control in many foods—a market presently dominated by soy protein isolates.

Several researchers have explored aqueous processing of oilseeds to extract oil and protein as a possible alternative to traditional organic solvent-based methods. Aqueous processes are environment-friendly, safe, and may require lower capital investment than solvent-based processes (4). A basic aqueous process for an oilseed consists of grinding, extraction, oil–protein separation, and drying to recover oil and protein. The key obstacle to the development of a viable aqueous process is the difficulty of oil–protein separation in aqueous media, where the native protein serves to stabilize the dispersed oil droplets. In the case of coconuts, centrifugation enables separation of the emulsion formed on extraction into clear oil and an aqueous phase containing protein (5). However, in the case of oilseeds, such as canola, soy, and mustard, a clean separation into clear oil and an aqueous phase is difficult to achieve. As a result, the protein, which is usually recovered by precipitation from the oil-containing aqueous phase, is bound to the oil, resulting in protein isolates that contain oil. The production of oil-free protein isolates using aqueous processing was the objective of this work.

Microfiltration (MF) membranes have been used for various protein separation and recovery applications in the dairy, food, and pharmaceutical industries. In particular, the use of cross-flow MF membranes to separate whey protein from milk fat and casein has shown that hydrophilic membranes may be used to separate constituents on the basis of both size and surface charge (6). The use of hydrophilic MF membranes to separate oil and protein extracted from mustard seed was investigated in this work. The oil-free protein solution thus obtained was then processed by UF and diafiltration (DF) to produce protein isolates that are low in antinutritional compounds. The process did not require the use of organic solvents at any stage.

It is well known that the fouling of MF membranes leads to the reduction of permeate flux and decrease in the apparent M.W. cut-off of these membranes, resulting in significant reduction in the permeation of protein. Protein fouling is a complex phenomenon involving the formation of a concentration polarization layer and the blocking of membrane pores due to protein adsorption (7). Several different approaches to mitigating the problem of protein fouling have been investigated, but none has proved universally successful. In our work, proteolytic enzyme treatment was considered as a method to increase protein passage through the membrane. In addition to increasing protein perme-

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ation, proteolytic enzyme treatment has been reported to improve the functional properties of food protein isolates (8).

EXPERIMENTAL PROCEDURES

Starting material. In the entire study, the same batch of dehulled, full-fat mustard (*S. alba*) flour obtained from G.S. Dunn & Co. Ltd. (Hamilton, Ontario, Canada) was used.

MF membranes. Hollow-fiber, polysulfone membranes (Millipore Corp., Bedford, MA), tubular ceramic membranes (TAMI Industries, St. Laurent, Québec, Canada) and flat-plate Durapore membranes (Millipore Corp.) made from polyvinylidenefluoride (PVDF) were initially screened for their oil and protein rejections and fluxes. Based on these tests, a 0.1-µm flat-plate Durapore membrane (0.1 m^2 in area) and a 0.1-µm hollow-fiber polysulfone membrane (0.03 m^2 in area) were selected for further study. The PVDF membranes were used in combination with a Pelicon-2 Tangential Flow Filtration (TFF) Unit (Millipore Corp.). The hollow-fiber membrane was tested by using an Amicon CH4 unit (Amicon Corp., Lexington, MA).

UF membranes. UF was used to concentrate protein while allowing low-M.W. compounds (e.g., phenolics, peptides) to pass through. A 10 kDa polysulfone (0.1 m^2 in area) flat-plate membrane cartridge (Millipore Corp.) was used in combination with a Pelicon-2 TFF unit. Pressure in the cartridge was controlled by a back-pressure valve at the outlet.

Definition of terms used. Concentration factor (CF) quantifies the extent to which the membrane processing has progressed (6). It is defined as

$$\mathrm{CF} = \frac{\text{volume of feed}}{\text{volume of retentate}} \quad [1]$$

Protein passage (%) is defined as the percentage of the total protein (N × 6.25) in the starting material that has been collected in the MF permeate at the end of the experiment:

$$\text{protein passage (\%)} = \frac{\text{protein in permeate (N} \times 6.25)}{\text{total protein in mustard flour (N} \times 6.25)} \quad [2]$$

The rejection coefficient R quantifies the permeability of a solute through the membrane (6). It is defined as

$$R = 1 - \frac{C_P}{C_R} \quad [3]$$

where C_P is the solute concentration in the permeate and C_R is the solute concentration in the retentate at any point.

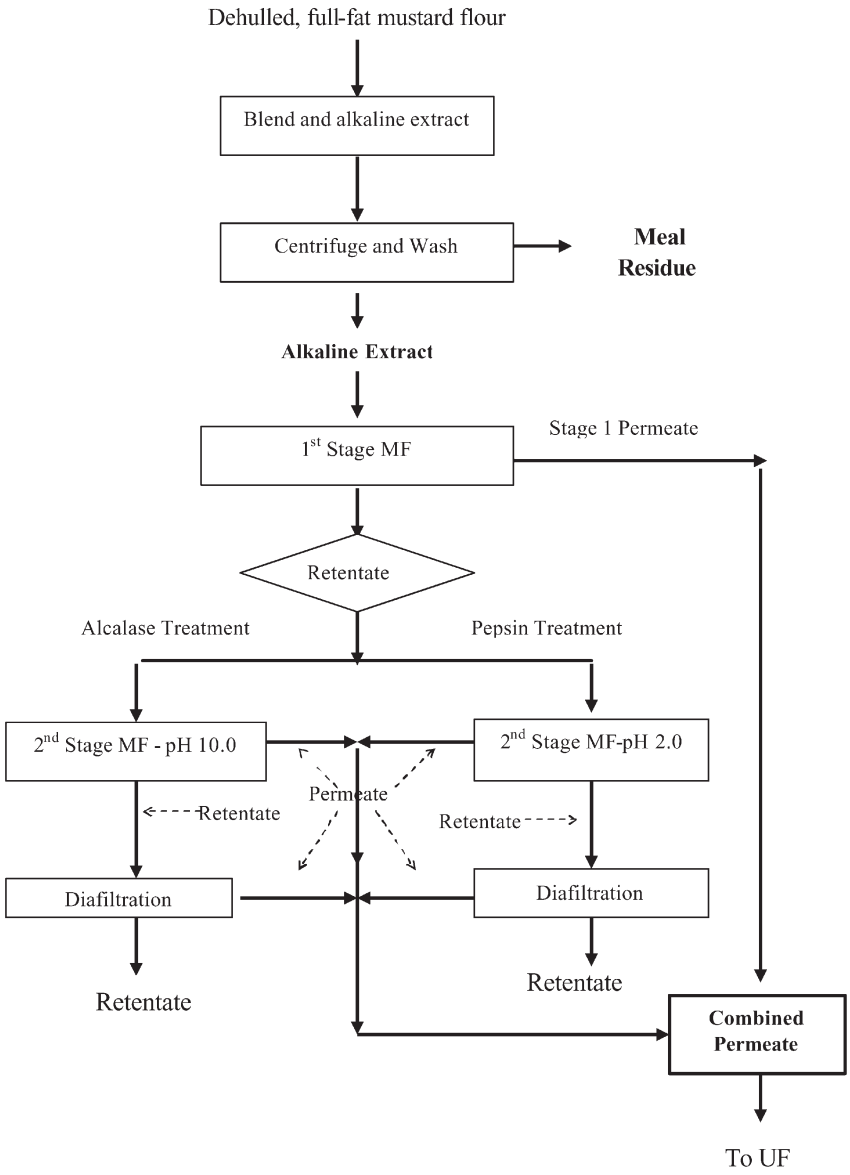
Protein determination. Protein (N × 6.25) was determined by the Kjeldahl method, American Association of Cereal Chemists method 46-12 (9), using a Büchi 425 digester and Büchi 315 distillation unit. All protein passage values reported in this paper represent the percentage of total protein in the flour that has permeated the membrane.

Electrophoresis. SDS-PAGE was performed by the method of Schagger and von Jagow (10). The samples were heated to 95°C for 5–10 min. The protein load applied was 20 µg per well. After loading, the gels were run at 200 V (constant voltage) for about 1.5 h. The gel system contained 0.1% (wt/vol) SDS and consisted of a 16.5% polyacrylamide separating gel (pH 8.5) and a 4% stacking gel (pH 8.5). The gels were stained in a 0.02% Coomassie blue solution and destained in a solution containing 40% methanol and 5% acetic acid. Broad-range-M.W. (6–175 kDa) protein markers (New England Biolabs, Beverly, MA) were used for M.W. estimation.

Basic MF-based process. Fifty grams of dehulled, full-fat mustard flour was blended for 30 s with 900 mL of water (1:18 solvent/meal ratio) in a commercial food blender. The protein and oil were extracted from the slurry at room temperature (25 ± 2°C) by maintaining the pH at 11 for 30 min using a pH-stat and 10% NaOH solution. The alkaline extract was centrifuged at 8,500 × g for 15 min. The meal residue was separated from the liquid extract and then washed twice with 300 mL (1:6 solvent/meal ratio) of water. The washing solution from the two wash stages was then combined with the original liquid extract. The entire extract solution (1.4 L) was prefiltered using Whatman No. 41 filter paper and then subjected to MF at a predetermined concentration factor. Samples were taken from the permeate at the end of the experiment to determine the amount of protein that had passed through the membrane.

Two-stage MF process. Permeate protein was recovered in two stages (Scheme 1). In the first stage, the alkaline extract made from 50 g of dehulled flour at pH 11.0 (see previous paragraph) was concentrated by a factor of 3.5, which reduced the volume of retentate to 400 mL. This was followed by a second stage that consisted of enzyme treatment of the retentate followed by MF and DF. Before the second-stage MF, the pH of the retentate was adjusted to maximize enzyme activity. With pepsin (EC 3.4.23.1, produced from porcine gastric mucosa), the pH of the retentate was brought down to 2.0. The required amount of pepsin (Sigma-Aldrich, Mississauga, Ontario, Canada) was dissolved in 50 mL of deionized water and added to the retentate solution to bring the volume up to 450 mL. The pH was maintained at 2.0 for 30 min by using 5 N HCl in a pH-stat in order to break down the large protein molecules and aggregates. The temperature was maintained at 37°C by immersing the beaker containing the retentate in a water bath. After 30 min, this treated solution was subjected to MF at CF = 3. The 150 mL of retentate solution remaining at the end of the second-stage MF was then diafiltered using a diavolume (DV) of 5 (750 mL) with water at pH 2.0. The pH of the permeate from this stage was slowly increased to 11.0 to terminate the enzyme reaction. It was then combined with the permeate from the first stage of MF.

Alcalase 2.4L (EC 3.4.21.62), a commercial food-grade endopeptidase (consisting mainly of subtilisin A) was also evaluated. The required quantity of Alcalase (Novozymes North America Inc., Franklinkton, NC) was added to 50 mL of deionized water and dissolved by using a magnetic stirring plate and stirring bar. The enzyme solution was added to 400 mL of retentate while the MF was in progress, bringing the volume up to 450 mL. The pH of the retentate decreased continuously after the Alcalase was added, signifying the progress of the proteolysis. It was allowed to drop to 10.0, where Alcalase shows appreciable activity, and then maintained at this pH using 1 N



SCHEME 1

NaOH stored in the pH-stat. This treated solution was concentrated by MF at CF = 3. The solution was then diafiltered at DV = 5 using aqueous NaOH at pH 10.0. The permeates from the two stages of MF were collected in the same beaker and the pH was increased to 11.0, where Alcalase shows zero activity.

Protein recovery. Protein was recovered from the combined permeate according to a process previously developed in our laboratory (2,3). A sufficient quantity of NaCl was added to the combined permeate to bring its concentration to 0.05 M. It was then heated to 60°C for 30 min, cooled to 40°C, and then ultrafiltered using a 10 kDa polysulfone UF membrane at CF = 5. The retentate at the end of UF was diafiltered at DV = 5. Both precipitated and soluble proteins were recovered from the retentate after DF. The pH of the retentate was lowered to 6.5 using a 1 N HCl solution, and the pH was maintained at this level for 15 min with further addition of 1 N HCl as required. The resulting suspension was centrifuged at 8,500 × *g* for 15 min. The supernatant was decanted and filtered using Whatman No. 41 filter paper. The precipitate was washed in the centrifuge tubes with five times its weight of water and centrifuged again at 8500 × *g* for 15 min. The original supernatant and washing liquid were then combined and freeze-dried using a Thermovac freeze-drier to produce a soluble protein isolate (SPI). The precipitate was freeze-dried to produce a precipitated protein isolate (PPI).

RESULTS AND DISCUSSION

Mustard protein profile. SDS-PAGE results showed that the alkaline protein extract from mustard (*S. alba*) ranged in size from ~6 to ~80 kDa (Fig. 1, lane 2). The M.W. of the proteins cluster in several regions or bands throughout the 6–80 kDa M.W. range with two major bands between 6 and 16 kDa, three distinct bands between 20 and 25 kDa, four bands

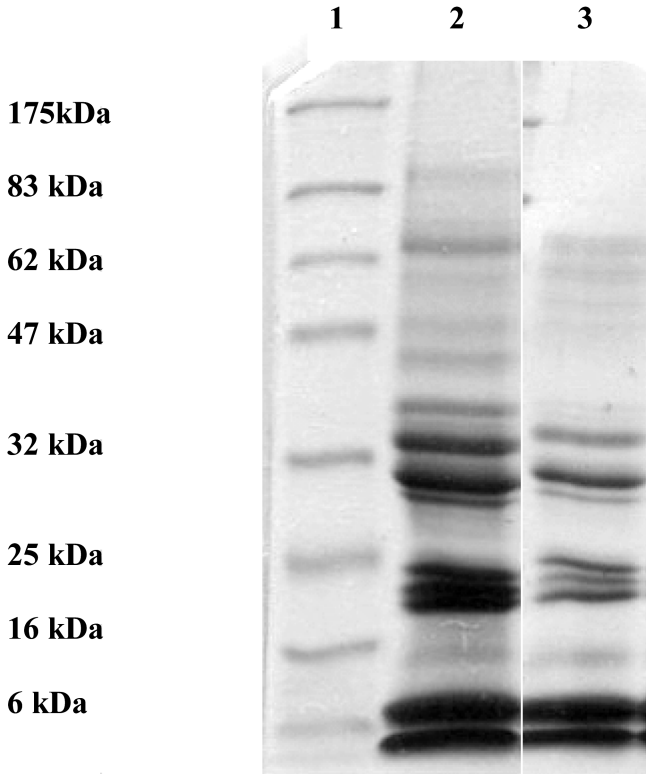


FIG. 1. M.W. profile of mustard protein using SDS-PAGE. Lane 1, standard marker proteins; lane 2, protein in alkaline extract at pH 11.0; lane 3, protein in microfiltration permeate.

clustered around 32 kDa, and one band around 62 kDa. The results also concur with the findings of Alanis-Guzman *et al.* (11) who reported that the largest amount of protein from *Brassica campestris* L. (wild mustard) is concentrated around bands of 20 and 36 kDa.

The results of SDS-PAGE indicated that it may be possible to separate the extracted protein and oil from mustard by using a hydrophilic MF membrane that will retain the oil and permeate the protein. The oil is retained by a combination of two mechanisms. Some of the oil is repelled by the hydrophilic surface of the membrane, whereas some of the dispersed oil in the emulsion exists in the form of large micelles that are rejected by the membrane on the basis of size. As the largest individual protein in mustard seed flour is less than 100 kDa in size and the pore size of a 0.1 μm membrane is an order of magnitude larger than 1000 kDa, the separation appeared feasible.

Accordingly, a 0.1-μm hollow-fiber MF membrane made of polysulfone was first tested for this purpose. The previously determined optimal conditions for alkaline extraction of rapeseed meal—water/flour ratio, extraction time, and number of washings—were applied to mustard meal (2). The meal was extracted at a pH of 12.0, where more than 90% of the protein in dehulled flour is soluble. When the alkaline extract was microfiltered, it was found that the MF permeate was transparent and clear while the retentate became progressively more viscous and opaque as the MF proceeded, showing that oil was being concentrated in the retentate. Further, oil was not detectable in the solid protein products that were recovered from the permeate. This showed that the membrane was successfully retaining the oil. However, results from an experiment conducted at CF = 4 indicated that only 17% of the total protein in the flour passed through the membrane. The bulk of the dissolved protein (~70% of the total protein) remained in the retentate along with the oil. When SDS-PAGE was used to compare the protein that permeated the membrane with the protein extracted from the flour (Fig. 1, lanes 2 and 3), nearly all the different kinds of protein initially present in the alkaline extract were found to have permeated the membrane. This indicates that there is no particular protein that is preferentially retained or permeated by the membrane. The retention of high amounts of protein despite this fact implies that a large amount of each protein may be present in aggregated form and is retained by the membrane while the rest of it exists in the free form and passes through the membrane.

In addition, fouling of MF membranes is known to lead them to reject protein even when their pore size is an order of magnitude larger than the size of the proteins (7). To determine the protein transmission characteristics, an experiment was performed to analyze protein passage as the MF proceeded. The concentration of the protein in the permeate remained fairly constant through the course of the MF, whereas the concentration of protein in the retentate increased continuously (Fig. 2). The large difference in concentration of protein between the permeate and the retentate is evident from the beginning of the process. This seems to indicate that protein is present in two distinct populations: one that appears to pass through the membrane freely at a rate proportional to the solvent flow rate, and another that is completely rejected by the membrane. This is consistent with some of the proteins being tied up in large molecular aggregates and oil-bodies that have typical sizes of 1–5 μm, which are much larger than the pore size of the membrane (12).

Effect of different treatments to increase protein transmission. Increased protein transmission could not be achieved simply by concentrating the retentate, so different treatments—both physical and chemical—were investigated in an attempt to increase protein passage through the MF membrane. The results of using these different treatments are shown in Figure 3.

Defatting. We initially suspected that the presence of oil in the emulsion could be contributing to the reduced passage of protein. When the alkaline extract prepared from defatted flour (hexane-extracted dehulled, full-fat flour) was passed through the membrane at CF = 4, 28% of the protein passed through the membrane (Fig. 3). Although increased protein passage was observed with defatted meal, 60% of the protein was still retained by the membrane.

Protein aggregates aggravate protein fouling, and several studies have shown that the removal of aggregates or the prevention of aggregation prior to membrane processing is beneficial (13,14). Some of these treatments were tested to mitigate the problem of aggregate formation and to reduce mem-

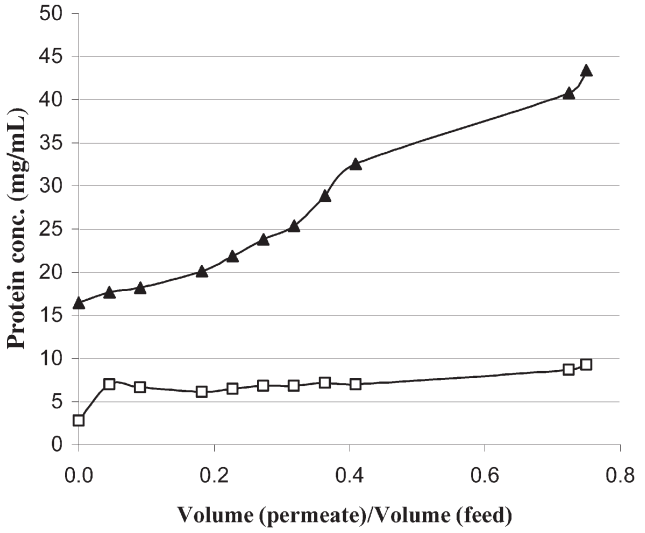


FIG. 2. Protein passage progress with increasing concentration factor using 0.1-µm polysulfone membrane. □, Concentration in permeate; ▲, concentration in retentate.

brane fouling (Fig. 3). All these treatments were tried on defatted meal to ensure that the presence of oil had no effect on the treatment.

Yeast treatment and surfactant treatment. The effect of yeast treatment on defatted meal protein passage was investigated according to the method of Kuberkar and Davis (13). However, the results from this treatment were not encouraging, as protein passage was not increased significantly at different concentrations of yeast cells (Fig. 3). Treatment with SDS was used to render the membrane more hydrophilic, thus preventing irreversible hydrophobic–hydrophobic linkages between the protein and the membrane surface and reducing fouling. However, this approach did not lead to improved protein transmission (Fig. 3).

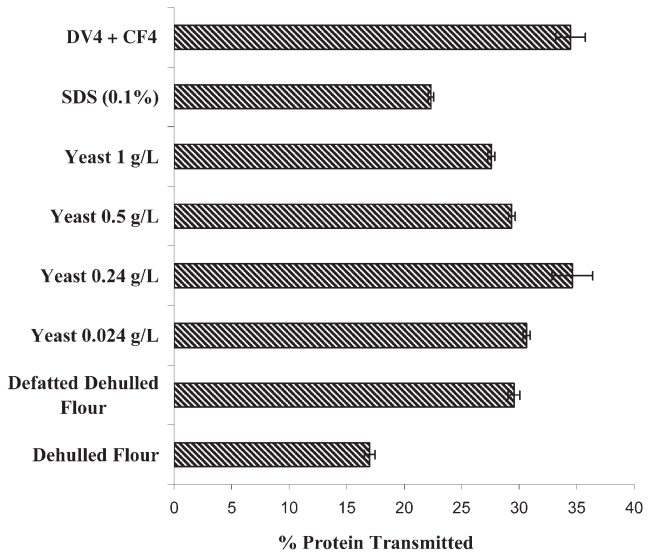


FIG. 3. Effect of different treatments on protein transmission: 0.1-µm polysulfone membrane; concentration factor (CF) = 4; diavolume (DV) = 4.

DF. The effect of DF after MF on defatted flour protein passage was studied. Theoretically, DF should lead to an exponential drop in the concentration of unaggregated protein. Results indicate that although it was helpful in significantly ($P < 0.05$) increasing the protein transmission for defatted flour from 28 to around 33% when a DV of 4 was used, the increase was not large enough to merit further attention. If 28% of the protein passes at CF = 4, then it represents 75% of the protein that passes freely. This implies that the total amount of protein that freely passes is 37%. By using a classical DF model (6) and assuming the protein passing freely to have $R = 0$ and the protein being retained to have $R = 1$, the amount of protein that is expected to pass through at DV = 4 after concentration by CF = 4 is 36.8% according to Equation 4,

$$\frac{C_R}{C_O} = e^{-\mathrm{DV}(1-R)} \qquad [4]$$

where C_R is the concentration of protein in the retentate at the end of DF and C_O is the concentration of protein in the retentate at the beginning of DF. This is in good agreement with the experimental value of 33% that was obtained and lends credence to the theory that ~65% of the protein is too large to pass through.

Effect of different membrane systems on protein passage. The failure of the preceding methods to increase protein passage beyond 35% led to the investigation of new membranes to improve protein transmission efficiency. The two alternative systems that were tried were tubular ceramic membranes and polymeric flat-plate membranes made of PVDF. All experiments were conducted at CF = 4 with brand-new membranes. The PVDF membrane clearly showed superior performance to the ceramic and polysulfone membranes (Fig. 4). PVDF membranes with 0.1-µm pores had the highest protein

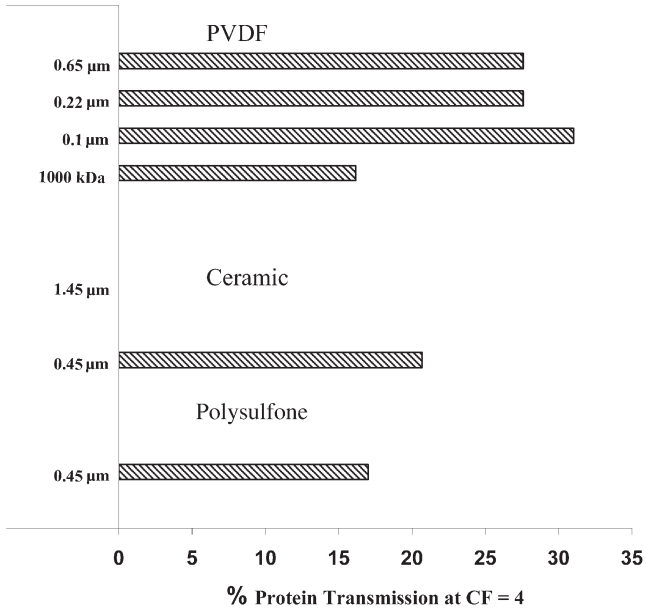


FIG. 4. Effect of different membranes on protein passage (CF = 4). PVDF, polyvinylidene fluoride; for other abbreviation see Figure 3.

transmission, with over 30% of the protein being transmitted. Increasing pore size did not contribute to increased protein passage. The 0.65- and 0.45-µm membranes showed lower protein passage than did the 0.1-µm membrane. Indeed, a larger pore size also contributes to an increased potential for fouling because the larger aggregates can now enter the pores and enmesh themselves within (7).

One particularly interesting result is the protein passage obtained for the 1.45-µm ceramic membrane. This number is not included in Figure 4 because the experiment could not be completed. The permeate flow from this membrane stopped completely within a few seconds of the commencement of the microfiltration, which indicated that the aggregates formed in solution are of the same order of magnitude in size as the membrane pores. It is postulated that the aggregates completely plug the pores, resulting in the total loss of flux. Based on these results, all further experiments were performed with 0.1-µm PVDF membranes.

Two-stage MF with proteolytic enzyme treatment. Because the methods discussed in the previous sections did not lead to transmission of more than 35% of the protein, proteolytic enzyme treatment was tested as an alternative. Nearly 25% of the native protein could pass through the 0.1-µm membrane at CF = 4, so we decided to permeate this protein first before breaking down the aggregated protein that remained in the retentate with proteolytic enzymes. Mustard protein was recovered in two stages (Scheme 1). A first-stage MF step recovered some native protein. It was followed by enzymatic treatment and a second-stage MF/DF step to recover further protein. The aim of enzymatic treatment was to break the large protein aggregates and molecules into sizes just small enough to pass through the membrane and not to hydrolyze them into small peptides. Enzyme treatment also has the potential to improve the functional properties of the final protein product (8). In preliminary experiments, enzyme pretreatment of the mustard flour was found to be less effective than two-stage MF in recovering intact protein. After screening different enzymes for their efficiency in the enzymic hydrolysis of mustard protein, pepsin and Alcalase were chosen for this study. Alcalase has the advantage of being used without changing the pH of the alkaline extract due to its activity at alkaline pH values, thus reducing the complexity of the process.

Protein passage in two-stage MF. Increased protein passage was observed after treatment with pepsin and Alcalase. Proteolytic enzyme treatment with pepsin increased the protein passage through the membrane from 25 to 65% (Fig. 5A). When Alcalase was used, the protein passage was increased to only 50% (Fig. 5B). The increased protein passage is attributed to the fragmentation of protein aggregates, leading to the permeation of more protein through the membrane. In addition, enzymatic treatment had no effect on the permeation of oil, which meant the oil–protein separation was still being accomplished.

Protein products from two-stage MF. The permeate from the process that we developed was subjected to an UF-based purification process, also developed in our laboratories (2,3), that reduces the concentration of glucosinolates, phenolics, and small peptides in the final protein product and simultaneously concentrates the protein in the retentate. Two oil-free protein products were recovered—an SPI and a PPI. In the case of both Alcalase and pepsin, the protein products were shown to be protein isolates that contained over 90% protein (N × 6.25) by weight (Tables 1 and 2). The protein recovered in the form of isolates at the end of the purification process accounted for 40% of the total protein in the case of pepsin and 25% in the case of Alcalase, in comparison to the less than 10% obtained without enzyme treatment. Oil recovery, however, was poor due to the difficulties encountered in recovering the oil from the retentate at the end of MF, which was in the form of an emulsion. Although protein recovery was significantly increased with the two-stage MF process, there were also higher protein losses during UF. Even without proteolytic enzyme treatment, ~10% of the total protein was lost due to the existence of small proteins and nonprotein nitrogen compounds that permeated the UF membrane. The greater loss of protein through the UF membrane with the enzyme-based process was due to the breakdown of some of the larger protein to polypeptides with M.W. below the cutoff of the UF membrane (10 kDa). In practice, such a treatment would use MF membranes coated with immobilized enzyme.

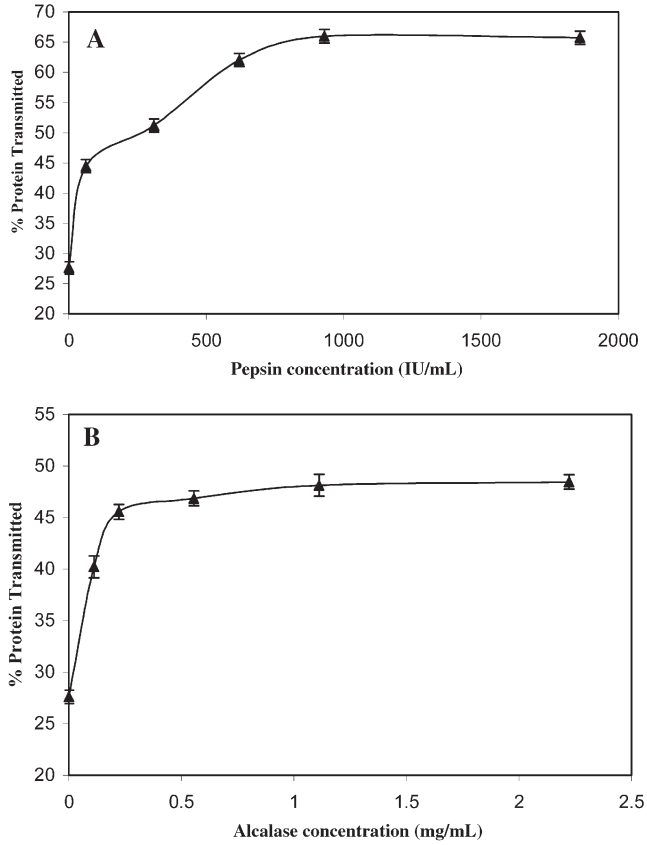


FIG. 5. Effect of enzyme concentration on protein passage (two-stage microfiltration, 0.1-µm PVDF membrane) with (A) pepsin and (B) Alcalase. For abbreviations see Figures 3 and 4.

TABLE 1
Protein, Oil, and Solids Distribution Among Products: Two-Stage Microfiltration with Pepsin

	Based on composition (%)		Based on yield (%)		
Product	% Protein[a] (N × 6.25)	% Oil[a]	% Solids	% Protein (N × 6.25)	% Oil
Mustard flour	33.6 ± 0.2	34.7 ± 0.4	100	100	100
Meal residue	18.6 ± 0.3	41.9 ± 1.2	35.5	18.7	40.7
PPI-retentate[b]	19.6 ± 0.3	74.5 ± 0.7	21.1	11.7	43.0
SPI-retentate[c]	75.6 ± 1.3	14.7 ± 0.8	3.1	5.6	0.5
PPI-permeate	100.6 ± 3.0*	0	2.6	8.7	0
SPI-permeate	90.8 ± 0.8	0	12.6	31.5	0
UF-permeate	0.1 ± 0	0	15.9	18.6	0
Losses			9.2	5.2	15.8

[a]On moisture-free basis.
[b]PPI, precipitated protein isolate.
[c]SPI, soluble protein isolate. (*) This value exceeds 100 due to the use of the standard conversion factor of 6.25.

TABLE 2
Protein, Oil, and Solids Distribution Among Products: Two-Stage Microfiltration with Alcalase

	Based on composition (%)		Based on yield (%)		
Product	% Protein[a] (N × 6.25)	% Oil[a]	% Solids	% Protein (N × 6.25)	% Oil
Mustard flour	33.6 ± 0.2	34.7 ± 0.4	100	100	100
Meal residue	20.3 ± 0.3	40.2 ± 0.5	33.7	19.8	37.1
PPI-retentate	30.6 ± 0.3	65.0 ± 0.4	21.6	18.7	38.3
SPI-retentate	54.5 ± 1.0	19.0 ± 0.5	3.2	5.0	1.7
PPI-permeate	102.5 ± 1.1*	0	2.5	7.3	0
SPI-permeate	91.7 ± 0.6	0	6.7	17.4	0
UF permeate	0.1 ± 0.0	0	23.3	25.0	0
Losses			9.0	6.8	22.9

[a]On moisture-free basis. For abbreviations see Table 1. (*) This value exceeds 100 due to the use of the standard conversion factor of 6.25.

This would reduce costs of the enzyme treatment and prevent excessive hydrolysis.

With the optimized enzyme process using pepsin, the two-stage MF process can recover over 60% of the protein in mustard flour in the oil-free permeate. The protein isolates recovered from the permeate accounted for 40% of the protein in the flour, and both the SPI and PPI contained over 90% protein. The meal residue, containing 20% protein, could be used in food or animal feed. The retentate from the MF contained 20% of the initial protein but also had close to 45% of the oil from the flour. Protein products recovered from the retentate also contained more than 80% protein on an oil-free basis. As this process leads to a 10-fold concentration of oil in the retentate, centrifugation of this oil-rich stream leads to the production of a clear aqueous layer and a creamy residue. Further work is necessary to determine how oil-free protein and oil can be recovered from this stream. This and enzyme costs will determine the viability of this process as a solvent-free approach to produce oil-free proteins from oilseeds.

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