International Journal of Applied Science and Engineering
2004. 2, 2: 127-135

Isolation of Yellow Mustard Proteins by a Microfil Tration-based Process

Narongechai Prapakornwiriya and Levente L. Diosady*

Department of Chemical Engineering and Applied Chemistry,
University of Toronto,
Toronto, Ontario, Canada M5S 3E5

Abstract: Under alkaline conditions (pH 12), approximately 90% of the protein was extracted from dehulled yellow mustard flour. The resulting aqueous emulsion was microfiltered using a polysulfone hollow-fiber membrane. A concentration factor of 3 at pH 10 was found to be suitable for microfiltration. A total of 86 % of the protein was recoverable in the form of isolates. Precipitated protein isolate (PPI) from the permeate was essentially free of oil and contained 97% protein (Nx6.25), accounting for 14% of staring protein. PPI from retentate had 20% oil and 71% protein (Nx6.25) (94% oil- and moisture-free basis), representing 64% of the staring protein. Some 8% of proteins were recovered as soluble protein isolates, while approximately 4% of the proteins remained in the meal residue and 7% in the emulsion.

Keywords: microfiltration; protein isolate; yellow mustard.

1. Introduction

Yellow mustard is also known as white mustard (*Sinapis alba* -an older botanical name). The seeds are about 3 mm in diameter and have several food and feed uses. Yellow mustard seed is primarily used as a condiment but some is crushed as a source of edible oil. It is a nutritious seed containing 28% to 36% protein (DeClercq and Daun, 1999). Due to its excellent fat and water binding properties, it is often used in processed meats. The use of yellow mustard as a protein source is limited by its sharp taste and colour.

Recently, the feasibility of producing protein isolates from yellow mustard has been established by Xu et al. (2003). Solvent extracted, oil free yellow mustard meal was used as raw material for a membrane-based protein isolation process. Alkaline extraction was used to maximize protein solubility while lowering the solubility of impurities, especially phytate. Ultrafiltration and diafiltration were used to concentrate and purify the protein extract, prior to precipitation of an isolate that had very low levels of undesirable components such as glucosinolates, phenolics and phytates.

Although high quality protein isolates were produced based on the process developed by Xu (2003), the processes required solvent extraction of the oil, which is both time and energy intensive. The potential adverse health effects and danger of flammable solvents, such as hexane, reduce the attractiveness of the process. Accordingly, in this study, we attempted to replace solvent extraction, with a membrane-based aqueous extraction process.

Microfiltration was used in an attempt to recover oil-free proteins from an aqueous

* Corresponding author; e-mail: diosady@chem-eng.utoronto.ca
Accepted for Publication: June 16, 2004

system containing emulsified oil and dissolved protein. Caviedes (1996) showed that oil-free canola protein isolates could be recovered from aqueous solution by membrane processing. In this study, we tested the hypothesis that the oil forms large micelles that would be retained by a microfilter, while allowing the passage of dissolved protein molecules, which could then be further purified and recovered.

2. Materials and methods

Dehulled yellow mustard flour used in this study was obtained from Hermann Laue Spice Co. Ltd., Uxbridge, ON, Canada. All reagents were of analytical grade supplied by VWR International, Mississauga, ON. Distilled water was used throughout this study.

2.1. Experimental techniques

To prepare yellow mustard protein isolate by microfiltration, in each run 20 g of dehulled yellow mustard flour was extracted for 30 min with 360 mL of distilled water at a pre-set alkaline pH. The extract was centrifuged to separate the meal residue from dissolved proteins using a B-22 centrifuge (International Equipment Co., Needham, MA) (6000×*g*, 20 minutes). The meal residue was washed twice with water at a solvent-to-meal ratio of 6 (V/W) for 10 minutes. The resulting solution was centrifuged and filtered using Whatman No. 541 filter paper for the separation of emulsion and solid. The washed meal residue was dried using a Labconco Freeze Dryer 5 for 48 hours. The combined solution from the alkaline extract and the washing solution was microfiltered using an Amicon CH4 concentrator equipped with a Millipore DIAFLO H5MP01-43 hollow-fiber membrane cartridge (Millipore Ltd., Billerica, MA.). The membrane had a pore size of 0.1µm and a membrane area of approximately 0.45 m^2.

The protein in the permeate was then precipitated by lowering the pH to 5.5 followed by centrifugation and filtration. The supernatant was analyzed for soluble protein content. The precipitated proteins were washed twice with 5 times the solid (wet basis) volume. The washed precipitated proteins were freeze dried.

The retentate was centrifuged and filtered before isoelectric protein precipitation. The pH of the filtrate was lowered to 6.5, and the precipitated protein was recovered by centrifugation and filtration, respectively. The precipitated proteins were washed, and freeze dried, whereas the supernatant was analyzed for soluble protein content. The dried products, meal residue, and the PPIs from permeate and retentate, were analyzed.

To determine the optimum extraction condition for protein extraction the pH of extraction solution was initially set at pH values between 10 and 13 with pH increments of 0.5 pH units and maintained constant by the addition of 0.1N NaOH. At the end of the 30 min extraction period, the slurry obtained was centrifuged, the solids were washed with 2x120 g of distilled water (R = 6) at the same pH for 10 min and then the slurry was centrifuged to recover dissolved protein. The liquid phases were combined and filtered. The combined extract and the undissolved solid meal residues were analyzed for protein.

In a series of experiments designed to determine the effect of processing conditions, the combined alkaline extract was microfiltered at a concentration factor of 2, 3, 4 or 5 at pH 12. In an effort to determine the optimum pH for microfiltration, the pH of the combined alkaline extract was adjusted to 9, 10 or 11 using 1N HCl and microfiltered at a concentration factor of 3. The permeate and the retentate were further processed to obtain protein isolates, as described above.

The effect of ionic strength was tested by adding NaCl to the alkaline extract at concentrations of 0.01, 0.05, or 0.10M. The pH of the extract was adjusted to pH 12, with a concentration factor of 3, which were found

to be the optimum conditions. The permeate and the retentate were further processed to obtain protein isolates, as described above.

2.2. Chemical analyses

Moisture content was determined gravimetrically according to AACC Method 44-15A (AACC, 1976). Crude protein (Nx6.25) was determined by the Kjeldahl method according to AACC Method 46-12 (AACC, 1976). A Büchi 425 digester and a Büchi 325 distillation unit (Büchi Laboratorium-Technik AG, Flawil, Switzerland) were used. The oil content was determined according to AOCS Method Ba-38 (AOCS, 1980). Samples were extracted with hexane using a Soxhlet extractor for 24 hrs. The oil was then recovered by a Büchi RE120 Rotavapor and dried at 105°C in a forced-air oven for 2 hours then weighed. Para-hydroxylbenzyl glucosinolate, the major glucosinolate of yellow mustard, was determined by the spectrophotometric method of Josefsson (1968). The free thiocyanate ion was measured colourimetrically, and the result was expressed as μmole of free thiocyanate ion, para-hydroxylbenzyl isothiocyante, per gram oil-free, moisture-free meal. Myrosinase used for glucosinolate analysis was prepared according to the method of Wetter and Youngs (1976). Phytate content was analyzed by the method developed by Naczk et al. (1986). Phytate content was calculated by multiplying the phytate phosphorus content by a factor of 3.55. Phenolic acids were determined by the method developed by Xu and Diosady (1997). Total phenolic acid was reported as total sinapic acid.

3. Results and discussion

The effect of pH on protein extractability is presented in Figure 1. The protein extractability increased rapidly, from 62% at pH 10 to 90% at pH 12. Further increase of pH had only a slight effect. The results confirmed the trend reported by Xu et al. (2003), with only a slightly increased solubility throughout the pH range tested. The presence of oil did not affect the protein extractability similarly to results reported for rapeseed meal by Caviedes (1996). Accordingly, extraction at pH 12 was used in subsequent experiments.

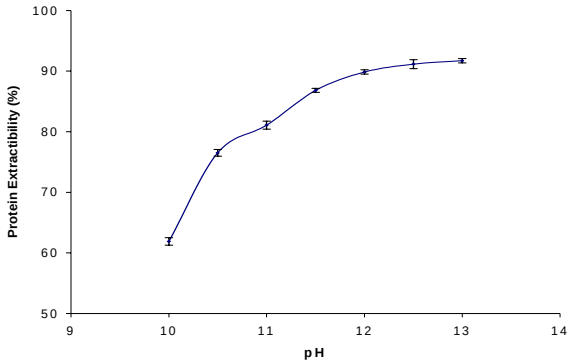

Figure 1. Protein extractability of yellow mustard

Figure 2 illustrates the protein recovery, protein content and oil content of PPI from the retentate. Increasing the concentration factor (CF) from 2 to 3 decreased protein recovery from 78 to 68% in the retentate, while increasing the protein content of the PPI from the retentate from 62 to 71%. Similarly, the oil content of PPI from the retentate decreased as CF increased. Increasing CF to more than 3 did not make a significant difference in protein recovery, protein content or oil content of the PPI from the retentate. Theoretically, the use of higher CF should remove most of the molecules of oil-free protein from the retentate. Increasing CF from 3 to 5 decreased protein recovery in the retentate only slightly, and the protein and oil content of PPI were not significantly changed. Increasing CF decreased the permeation flux due to increased viscosity and membrane fouling.

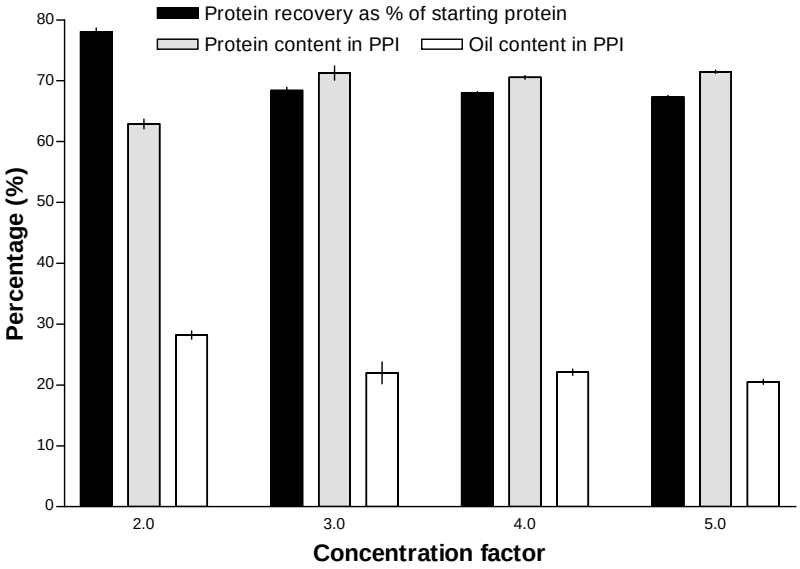

Figure 2. The effect of concentration factor on RT/PPI

The results indicate that oil did not pass through the microfilter, resulting in oil-free protein isolates in the permeate. Unfortunately, only a small fraction of the protein passed through the membrane. As shown in Figure 3, increasing CF from 2 to 3 increased protein recovery in the permeate from 10 to 22%, but further increasing CF had limited effect on protein permeation as most of the extracted protein was bound to oil in micelles and oil bodies.

The protein permeation through the microfilter was decreased as the pH of the extract was decreased before microfiltration. Approximately 5% less protein was recovered in the permeate as pH was changed from 12 to 9. The decrease of protein permeability was due to increase the hydrophobicity of the protein at lower pH values. The number of binding sites that could interact with oil increased and, in turn, the protein could more readily combine with oil in the extract. In this study, pH between 10 and 12 provided the highest protein recoveries in the permeate (19-22%) (Figure 4); however, analysis of the PPI from the retentate indicated that oil content in the PPI was minimized at pH 10 (20%), and thus this pH was used for further studies.

Ionic strength also had an effect on protein permeability through the microfilter. The protein recovery in the retentate, as well as the protein and oil content of the PPI increased slightly as NaCl concentration, and thus ionic strength was increased. The range of protein recovery, protein content and oil content were 68-73%, 68-71% and 21-24%, respectively. However, the protein recovery and purity in the PPI from the permeate were typically decreased by adding NaCl. The conformational changes of the protein molecules in the bulk solution or the adsorption of proteins to the membrane at increased ionic strength was reported earlier by Ehsani (1996). Increasing ionic strength tended to decrease the solubility of proteins causing less protein permeation through the membrane.

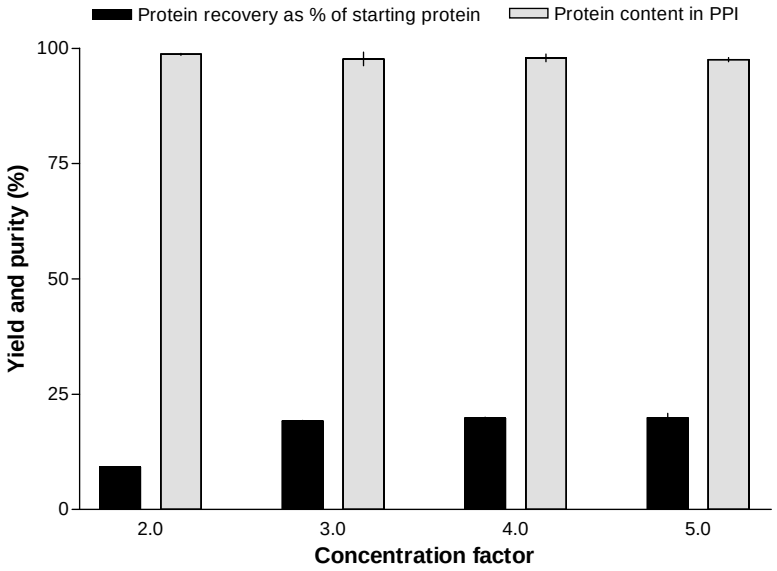

Figure 3. The effect of concentration factor on yield and purity of PM/PPI

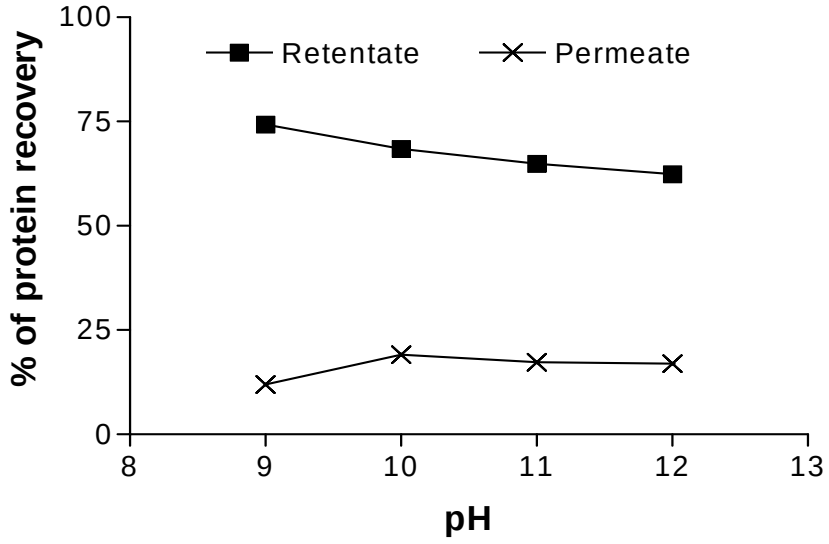

Figure 4. The effect of pH on protein recovery

The most effective operating conditions were employed in later tests. The product distribution obtained by the process is shown in Table 1. While most of the extracted proteins remained in the retentate (69% of starting protein), some 20% of the starting protein passed through the membrane. (The rest of the protein ended up in the meal residue (4%), in emulsion I (4%) and the rest (3%) was lost in sample manipulation.)

Table 1. Product distribution

Process stream	Protein		Oil	
	Recovery (%)[a]	Concentration (%)	Recovery (%)[a]	Concentration (%)
Mustard flour	-	34.5	-	32.44
Meal residue	4.12	9.20	29.32	34.81
Emulsion I	4.67	-	27.40	-
Retentate:				
Emulsion II	2.26	-	24.54	-
RT/PPI	64.43	71.26	17.16	20.18
Soluble protein	2.14	-	-	-
Permeate:	14.15	97.69	ND	ND
PM/PPI	5.30	-	-	-
Soluble protein	2.93	-	1.58	-
Losses				

All results are means of triplicates
[a] Protein recovery as % starting material
ND: Not detectable

Approximately 70% of protein passing through the membrane was recovered as the PPI, representing some 14% of the protein in the flour. The PPI obtained from the permeate was a of high purity (~97%), and was oil-free. Its colour was light brown and its taste was slightly astringent.

Approximately 69% of protein from the starting material was retained in the retentate and approximately 93% of these proteins were recovered as the PPI. The PPI contained approximately 94% protein (oil- and moisture-free basis) and 20% oil. Colour of this PPI was light brown and its taste was slightly astringent, and greasy due to the presence of 20% oil. The presence of oil in the retentate was due to the binding of the proteins to the oil in micelles and oil bodies, which had a typical size of 1μm.

In the process described in Figure 5, both the retentate and permeate contains some unprecipitated protein. The process to recover such soluble proteins was developed by Xu et al. (2003). However, the quantity of these proteins did not warrant the further processing required for recovering them as soluble protein isolates.

Approximately 4% of the protein in the starting material remained in the meal residue and some 7% in total was contained in the two emulsions. Non-protein nitrogen, and handling losses accounted for less than 3% of the starting protein.

More than 50% of the oil was recovered as emulsions and around 30% of the oil was retained in the meal residue. Furthermore, 17% of the oil was recovered as the PPI from the retentate. Evaluation of the oil-free solid content of the emulsion was difficult. As a result, in this study little attention was paid to further processing of the two emulsions, even though we realize that an industrial process will have to either use these emulsions, or must break them to recover the oil to be economically viable.

The recovered products, including meal residue, PPIs from permeate and retentate, were further analyzed for undesirable components such as glucosinolates, phytates and phenolic compounds.

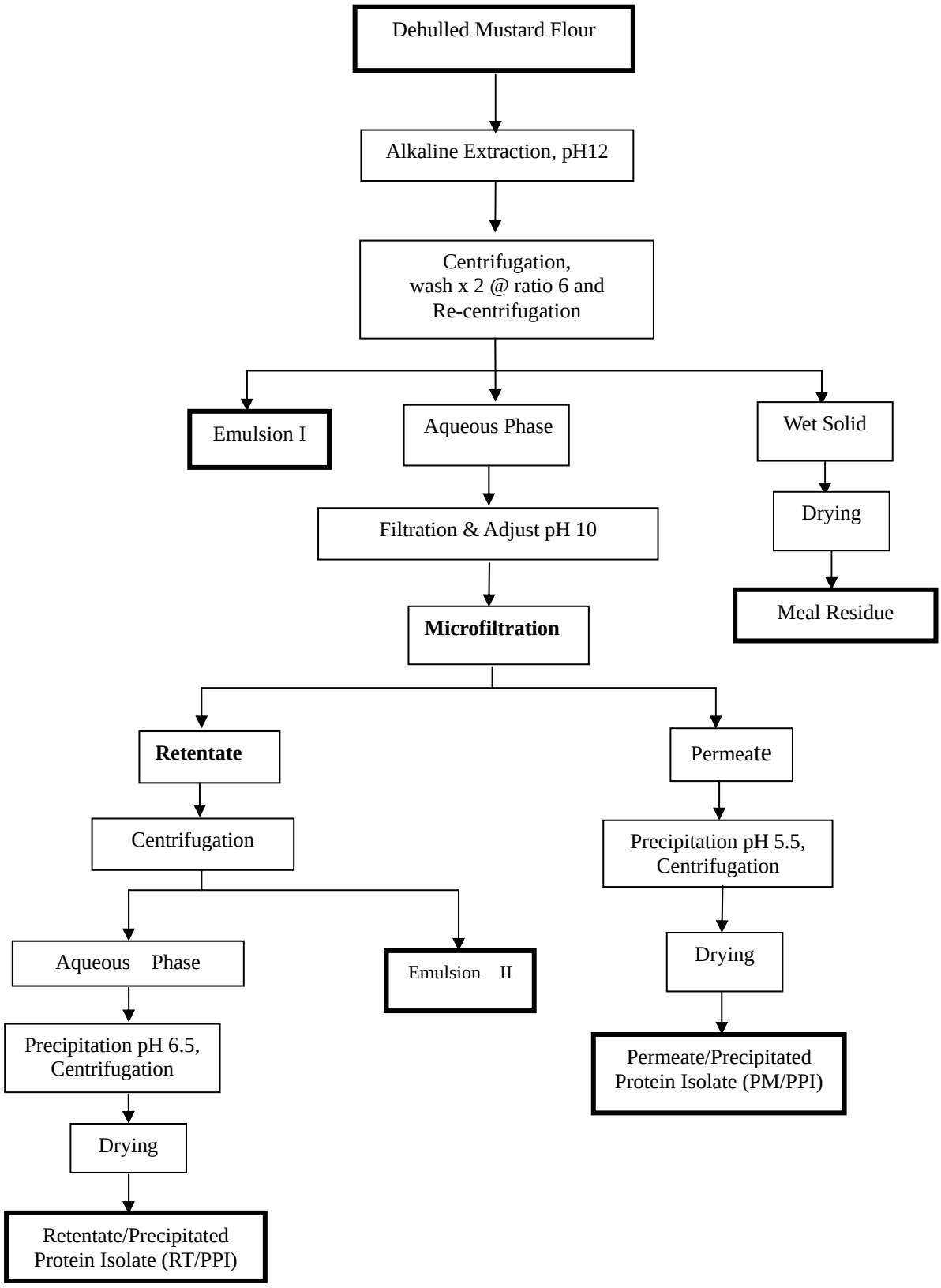

Figure 5. Microfiltration-based process for producing yellow mustard protein isolates

Glucosinolates were undetectable in all protein products (< 2.2 μmole/g). High concentrations of phenolic compounds were found in precipitated protein isolates from both the retentate (2385 mg/100g of sample) and the permeate (1243 mg/100g of sample). This was probably responsible for the astringent taste of precipitated protein isolates. These phenolic compounds would be probably removed by membrane processing as described by Xu and Diosady (2002).

The concentration of phytate was low in PPIs in both the permeate and the retentate. Although the phytate content of the starting material was considerable, most of the phytates were retained in the meal residue (Table 2), as their alkaline solubility is low (Tzeng et al. 1989).

Table 2. Product evaluations

Product	Glucosinolate[a] (μmole/g sample)	Phenolic compound[a] (mg/100g sample)	Phytate[b] (%)
Mustard flour	198	1245	2.78
Meal residue	< 2.2	164	3.42
RT/PPI	< 2.2	2385	0.09
PM/PPI	< 2.2	1243	0.10

All results are reported as means of triplicates
[a] Moisture- and oil-free basis
[b] Moisture-free basis

In order to have the re-ordered part processed as early as possible, the part will move back only one position. If the second lineup matches again, then both parts will move back one position, and so on. If there are *n* consecutive matches, then the mechanism will insert a bubble at the entrance. If one bubble still causes deadlock, then insert another, and so on, until no matches happen. The number of *n* can be determine by the product due day, and will not be elaborated in this paper.

Evidently, the more insertion of bubbles, the longer overall delay will be expected. This is also part of the characteristic relationship between the cell and parts. The following section will introduce other dispatching rules to improve the relationship. The aim is to cut down total number of deadlock patterns, so that the probability of hitting a match is minimized.

4. Conclusions

The use of microfiltration to separate proteins from the aqueous extract of dehulled yellow mustard flour was shown to be technically feasible. Under the best conditions tested the precipitated protein isolate from the permeate accounted for 14% of the starting protein, with 97% protein content (moisture-free basis). It was low in phytates and essentially free of glucosinolates. The corresponding precipitated protein isolate from the retentate recovered approximately 64% of the starting protein and contained 71% protein and 20% oil, (94% protein, oil- and moisture–free basis). Although the microfiltration recovered high quality protein isolates without the use of an organic solvent, most of the extracted oil was recovered in the form of stable emulsions. The practical application of this process will require either a technique to recover oil and protein from these emulsions, or the development of a valued use for the emulsions.

Acknowledgment

The authors would like to thank Hermann Laue Spice Company of Canada, Materials & Manufacturing, Ontario (MMO), and the Natural Sciences and Engineering Research Council of Canada (NSERC) for their financial and material support.

References

[1] DeClercq R. D., and Daun, J. K. 1999. Quality of Western Canadian canola. Grain Research Laboratory. Canadian Grain Commission. http://www.cgc.ca.

[2] Xu, L., Lui, F., Luo, H., and Diosady, L. L. 2003. Production of protein isolates from yellow mustard meals by membrane processes. *Food Research International*, 8, 36: 849-856.

[3] Caviedes, J. 1996. "*Aqueous Processing of Rapeseed (Canola)*". Master of Applied Science Thesis. Department of Chemical Engineering and Applied Chemistry. University of Toronto, Toronto, Ontario, Canada.

[4] American Association of Cereal Chemists. 1976. "*Approved Methods of the American Association of Cereal Chemists*". 7th Edition, American Association of Cereal Chemists, St. Paul, Minnesota, U.S.A.

[5] American Oil Chemists' Society. 1980. "*Official and Tentative Methods of the American Oil Chemists' Society*". 3rd Edition, American Oil Chemists' Society, Champaign, Illinois, U.S.A.

[6] Josefsson, E. 1968. Method for quantitative determination of p-hydroxybenzyl isothiocyanate in digests of seed meal of *Sinapis alba*. *Journal of Science and Food Agriculture*, 19: 192-194.

[7] Wetter, L. R. and Youngs, C. G. 1976. A thiourea-UV assay for total glucosinolate content in rapeseed meals. *Journal of American Oil Chemists' Society*, 4, 53: 162-164.

[8] Naczk, M., Diosady, L. L., and Rubin, L. J. 1986. The phytate and complex phenol content of meals produced by alkanol-ammonia/hexane extraction of canola. *Lebensmittelwissen and Technogie*, 1, 19: 13-16.

[9] Xu, L. and Diosady, L. L. 1997. Rapid method for total phenolic acid determination in rapeseed/canola meals. *Food Research International*, 8, 30: 571-574.

[10] Ehsani, N. 1996. Literature study in characterization and fractionation in ultrafiltration of proteins. In N. Ehsani "*A Study of Fractionation and Ultrafiltration of Proteins with Characterized Modified and Unmodified Membranes*". Lappeenranta University of Technology, Finland.

[11] Xu, L. and Diosady L. L. 2002. Removal of phenolic vompounds in the production of high-quality canola protein isolates. *Food Research International*, 1, 35: 23-30.

[12] Tzeng, Y-M., Diosady, L. L., and Rubin, L. J. 1990. Production of canola protein material by alkaline extraction, Precipitation and membrane processing. *Journal of Food Science*, 4, 55: 1147-1156.