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Food Chemistry 65 (1999) 189–194

Food Chemistry

Functional properties of soy protein hydrolysate produced from a continuous membrane reactor system

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Received 2 June 1998; received in revised form and accepted 20 August 1998

Abstract

Two types of soy protein hydrolysates were produced by hydrolysis of isolated soy protein (ISP) with a combination of Alcalase and Flavourzyme in a continuous membrane system with 3000 and 30,000 daltons molecular-weight-cut-off (MWCO) membranes, respectively. Both hydrolysates were completely soluble over a pH range of 2–9. Their water-binding capacity increased 1.8–3.4 times at a water activity of 0.6–0.95 as compared to intact ISP. The antioxidant activity of ISP was remarkably enhanced by enzyme hydrolysis. The hydrolysate from the 30,000 daltons MWCO membrane had a much higher antioxidant activity than that from the 3000 daltons MWCO membrane. The results suggested that both selected enzymes and MWCO membrane governed the functional properties of protein hydrolysates. Long-term operation study showed that the membrane reactor system could maintain a steady production of ISP hydrolysate over 16 h. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Protein hydrolysates have been employed to provide nutrients for individuals who experience difficulties in the digestion of intact protein (Mahmoud, Malone, Cordle, & 1992). The functional and immunological properties of proteins can be improved by modification and thus the modified proteins can be used in food systems as additives for beverage and infant formulae, as food texture enhancers, or as a pharmaceutical ingredient (Chiang, Cordle, & Thomas 1995; Mannheim & Cheryan, 1990).

Compared to acid or alkali hydrolysis, enzymatic hydrolysis of protein, using selective protease, provides milder process conditions and little or no undesirable side reactions or products. In addition, the final hydrolysate, after neutralization, contains less salt and the functionality of the final product can be controlled by selection of specific enzymes and reaction factors (Cheryan & Deeslie, 1983; Deslie & Cheryon 1988).

Conventional batch-type protein hydrolysis has several disadvantages such that it is (1) time-consuming and (2) labour-intensive, (3) it occupies a large floor space, (4) it involves high cost of enzymes which cannot be reused, (5) it gives nonhomogeneous products and fractions of varying molecular weight, (6) it is a slow process, (7) it causes inhibition of enzyme activity by the end product, (8) it gives low yields and productivity, (9) there is inactivation of enzyme by pH adjustment or heat treatment at the end of the reaction. (Deeslie & Cheryan, 1988; Lin ,Chiang, Cordle, & Thomas, 1997; Xavier, Goncalves, Moreva, & Corrondo, 1995).

In order to overcome these problems, an ultrafiltration membrane reactor has been developed and the major advantages are: (1) the enzyme can be repeatedly used and (2) the molecular size of the product can be governed by appropriate selection of the ultrafiltration membrane (Deeslie & Cheryan, 1981). Membrane reactors have been successfully used in the continuous productions of protein hydrolysate (Cheryan & Deeslie, 1983; Deeslie & Cheryon 1988; Chiang , Cordle, & Thomas, 1995), glucose syrup (Sims & Cheryan, 1992), milk protein (Mannheim & Cheryan, 1990), and β-casein/chymosin hydrolysate (Bouhalab , Modle, & Lenil 1993).

This paper reports a study of long-term steadiness of continuous operation of an ultrafiltration membrane reactor for producing soy protein hydrolysate and the functional properties of the protein hydrolysate in food systems.

2. Materials and methods

2.1. Materials

POMP® Isolated soy protein (ISP) was from the Union Chemical Ind. Co. (Taipei, Taiwan). Commercial

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PII: S0308-8146(98)00193-9

enzymes, Flavourzyme Type A and Alcalase 2.4 L, were purchased from NOVO Industri A/S (Copenhagen, Denmark). HPLC standards including cytochrome C, aprotinin, gastrin I, substance P, vitamin B12, and glycine were purchased from Merck (Darmdstadt, Germany) or Sigma Chemical Co. (St. Louis, MO). Polysulfone hollow fibre (UFP-30-C-9A) was from A/G Technology Corporation (Needham, MA). Refined soybean oil was obtained from I-Hua Company (Tainan, Taiwan). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were purchased from Gemfont Company (Taipei, Taiwan).

2.2. Batch reactor

The batch reactor consisted of a reaction vessel (10 liter) with steam jacket and stirring arm to provide uniform heating and mixing. Five to ten litres of 3% ISP solution were introduced into the reaction vessel and heated. Before the temperature reached 50°C, a 2.5% enzyme mixture (Flavourzyme:Alcalase = 3:2)/substrate (w/w) was added to the reaction vessel.

2.3. Continuous hollow fibre (HF) membrane reactor system

A 3% ISP solution prepared by vigorously stirring and heating was passed through a 100-mesh sieve to remove large particles. The reaction vessel (Fig. 1) was filled with the desired volume of 3% of ISP filtrate and the temperature of the tank was maintained at 50°C. A 2.5 % solution of enzyme mixture (Flavourzyme:Alcalase = 3:2)/substrate (w/w) was added to the soy protein filtrate. Inlet pressure and flow rate were adjusted appropriately. The reaction mixture was pumped to the hollow-fibre membrane where the large particles such as intact proteins or enzymes which could not penetrate the pores of the ultrafiltration membrane would recycle to the reaction vessel. The permeate, containing particles small enough to penetrate the membrane, was collected and lyophilized. The level of reaction mixture in the vessel was controlled by adjusting the flow rates between the ISP solution from the feed tank and the permeat from the membrane.

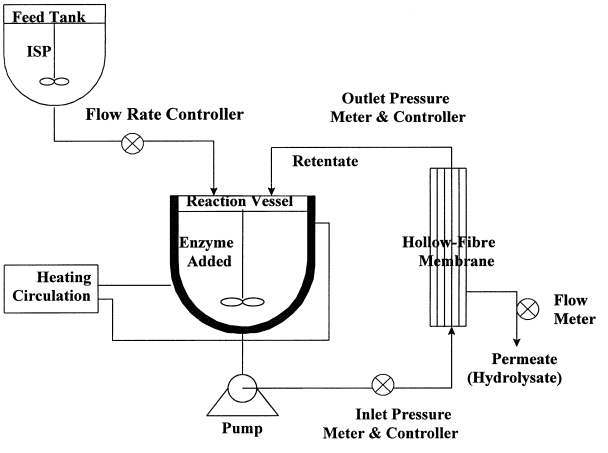

Fig. 1. Schematic diagram of continuous hollow-fiber membrane system.

2.4. Total nitrogen

Total nitrogen was measured by the micro-Kjeldahl method (AOCS, 1989), using a KjelTec system 1026 with rapid distillation unit (Tecator, Sweden). All data were corrected for nonprotein nitrogen (NPNi), which was determined as the initial nitrogen of ISP soluble in 10% trichloroacetic acid (TCA). The TCA-soluble nitrogen of the product (NPNp) was also determined. The % TCA-soluble nitrogen or % conversion (X) was expressed as

$$X = \frac{\mathrm{NPNp} - \mathrm{NPNi}}{TN - \mathrm{NPNi}} \times 100$$

where TN = total nitrogen in the product.

2.5. Productivity study

For the batch reactor, productivity was defined as

$$P_{\mathrm{batch}} = \frac{XTN_i}{E}$$

where E = enzyme concentration (mg/ml); TN_i = total nitrogen in unhydrolyzed substrate corrected for NPNi.

For the continuous HF membrane reactor, productivity was expressed as

$$P_I = \frac{PJt}{EV}$$

or

$$P_c = \sum P_I$$

where P_I = instantaneous productivity; P = average product output (mgN/ml) in a time period t (min); J = flow rate (ml/min); E = enyzme concentration (mg/ml); V = substrate volume (l); P_c = cumulative productivity. P_c was calculated over all time periods studied. The unit of productivity was mass hydrolysate/mass enzyme (mg N/mg enzyme). A more complete description of productivity was given by Cheryan and Deeslie (1983).

2.6. Molecular weight distribution

The molecular weight distribution of the hydrolysate was analyzed by high-performance size-exclusion chromatography (HPSEC). The HPSEC was equipped with

a Superdex HR 10/30 column, connected to a UV detector set at 214 nm. The mobile phase was 0.02 M phosphate buffer (pH 7.2) containing 0.25 M NaCl and flow rate was set at 0.25 ml/min.

2.7. Amino acid composition

Free amino acids of ISP and its hydrolysates were determined by a Beckman System 6300 High Performance Amino Acid Analyzer (Beckman Instrument, Inc., CA). Amino acid composition and contents of ISP and its hydrolysates were determined by a Hitachi L-8500 amino acid analyzer (Hitach, Ltd., Tokyo, Japan) using the method described by Simpson et al. (1976).

2.8. Solubility

The solubility of samples was measured by Nitrogen Solubility Index (AOCS, 1989). Thirty milliliters of distilled water were added slowly to a 400 ml beaker containing 2.5 g of sample and the solution was stirred with a glass rod. The desired pH of sample solution was adjusted using 2N NaOH or HCl and then brought to the final volume of 100 ml. After the sample was placed in a water bath for 2 h at 30°C and stirred constantly, the solution was centrifuged at 3000 rpm for 10 min. The supernatant was decanted and passed through a Whatman #4 filter paper. The filtrate was used for measuring the total nitrogen content. The solubility was calculated as:

$$\%\text{Solubility} = \frac{TNs}{TNi} \times 100$$

where *TNi* and *TNs* are the amounts of the total nitrogen of initial 2.5% sample solution and its filtrate, respectively.

2.9. Moisture sorption

Moisture sorption of samples was determined according to Lang, McCune, and Steinberg (1981). Mini-desiccators were employed to determine the water adsorption isotherm of samples at 22°C. Each desiccator contained 500 ml saturated salt solution in the base. One-gram samples were weighed in triplicate into standard weighing dishes. After incubation in a desiccator for 6 days, the weight gain of each sample was measured every day until its weight gain reached equilibrium.

2.10. Antioxidative activity

The method of AOCS cd 12b-92 was used to determine the antioxidative activity of ISP and its hydrolysates, using an oxidative stability instrument (Archer–Daniels–Midland Co., Notts, UK). Various concentrations of samples were added to sample tubes containing 5 g of soybean oil each. Temperature was set at 120°C and air pressure was controlled at 5.5 psi. Volatile material produced during oil oxidation was transferred to the Lexan conductivity cell with 50 ml of water and conductivity sensor, which connected to a computer. The computer would record the conductivity change and automatically calculate the OSI value.

3. Results and discussion

3.1. Continuous hollow fibre (HF) membrane reactor

Productivity and long-term operating stability of continuous HF membrane reactor were evaluated using a 30,000 daltons molecular-weight-cut-off (MWCO) membrane.Fig. 2 shows the comparison of productivity between batch reactor and continuous reactor as a function of volume replacement. The productivity of the batch reactor was independent of volume replacement, whereas that of the continuous membrane reactor had a linear increase as volume replacement increased. The result showed that reusing the enzyme has the advantage of higher productivity in the continuous membrane reactor. In batch reactors each volume replacements requires recharge with the same amount of enzyme. However, in the continuous HF membrane reactor, enzyme charge was done only at the beginning of the run. After two volume replacement the continuous reactor had a higher productivity than the batch reactors. This result also agreed with the findings of Mannheim and Cheryan (1990) and Chiang et al. (1995). They produced casein hydrolysates by a continuous membrane reactor and concluded that the reuse of enzyme resulted in a higher productivity as compared with a batch membrane reactor.

Long-term operating stability of the reactor was determined by hydrolysis of 3% (w/w) ISP with 2.5% (w/w of ISP) enzyme at 50°C (Fig. 3). The continuous HF membrane reactor showed a steady production of

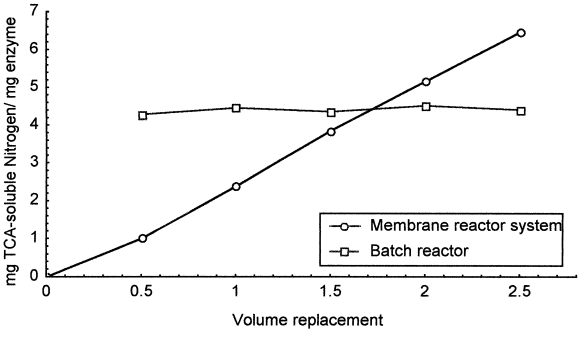

Fig. 2. Comparison of productivity between membrane reactor and batch reactor. Results are mean values of two replicates. Standard deviations did not exceed 2% of the recorded values.

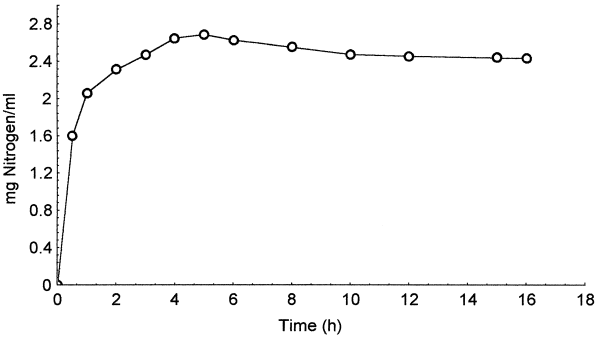

Fig. 3. Long-term operating stability of the continuous membrane system. Results are mean values of three replicates. Standard deviations did not exceed 2.5% of the recorded values.

protein hydrolysates for at least 16 h. Slight decrease in the product output after 8 h of hydrolysis reflected leakage of the enzyme. Enzyme leakage was confirmed by detecting residual enzyme activity in the permeate (data not shown). However, this could be compensated by dosing the reactor with fresh enzyme periodically. Iacobucci, Myers, Emi, and Myers, (1974) studied the continuous production of soybean protein hydrolysate in a constant flux membrane reactor. They concluded that the enzyme had to be replaced at a rate of 2% of the enzyme in the reactor per h in order to compensate for the loss of enzyme by permeation through the membrane. Chiang et al. (1995) also reported loss of the enzyme in a continuous membrane reactor. In order to maintain the long-term operating system for more than 16 h, periodic addition of fresh enzyme into the reactor was suggested. However, the dosage of fresh enzyme was not determined in the study.

3.2. Chemical characteristics of protein hydrolysates

Hydrolysates A and B were produced from the continuous HF membrane reactor using 3000 and 30,000 daltons MWCO membranes, respectively. Amino acid compositions of ISP and its hydrolysates are listed in Table 1. Glutamic and aspartic acids were the major amino acids in ISP and both hydrolysates. The difference in total amino acid composition was small between hydrolysates A and B. Based on HPLC analysis, the total free amino acid contents of ISP, hydrolysate A and hydrolysate B were 0.07, 11.0 and 12.1% (g free amino acid/100 g protein), respectively.

Both hydrolysates (A and B) had the same profile (data not shown) and were primarily composed of amino acids and small peptides with molecular weight <4000 daltons (Table 2). The major differences between hydrolysate A and B were the peptide content in the 500–2000 and 3000–4000 dalton fractions. The peptide content of hydrolysate A was 7% less in the 500–2000 dalton fraction and 10% more in 3000–4000 daltons fraction than that of hydrolysate B.

Table 1
Amino acid composition of ISP and its hydrolysates

Amino acid	Amount (g/100 g sample)		
	ISP	Hydrolysate A	Hydrolysate B
Alanine	3.52	3.78	3.59
Arginine	6.58	6.22	6.50
Aspartic acid	10.1	10.8	10.3
Cystine	8.95	6.06	7.43
Glutamic acid	17.6	12.7	13.0
Glycine	3.46	3.49	3.55
Histidine	2.08	1.76	1.97
Iosleucine	3.91	4.24	4.06
Leucine	7.07	7.59	7.05
Lysine	5.28	5.36	5.68
Methionine	0.97	0.91	0.89
Phenylalanine	4.69	5.20	4.85
Proline	4.50	4.24	4.65
Serine	4.42	4.96	4.21
Threonine	3.04	3.27	3.07
Trytophan	0.34	0.31	0.36
Tyrosine	3.23	3.53	3.19
Valine	3.75	3.98	3.84

Table 2
Molecular weight distribution of hydrolysates (%)

M.W. (daltons)	Hydrolysate A	Hydrolysate B
<500	13.4	15.4
500–2000	34.5	41.4
2000–3000	17.2	16.9
3000–4000	33.3	23.0
>4000	1.78	3.34

3.3. Functional properties of protein hydrolysates

3.3.1. Solubility

Fig. 4 shows the solubility profiles of ISP and its hydrolysates as a function of pH. Intact ISP had the highest solubility at pH 9 but very low solubility between pH 4 and 5. However, for hydrolysates A and B at the 4% level, both hydrolysates were easily and completely soluble or dispersible ($>99\%$) between pH 2 and 9. The enhanced solubility of the hydrolysates was due to their smaller molecular size (Turgeon, Gauthier, & Paquin 1992) and the newly exposed ionizable amino and carboxyl groups which increased the hydrophilicity of the hydrolysates (Phillips & Beuchat, 1981). According to Mahmoud (1994) and Frokjaer (1994), the hydrolysate with a wide range of pH solubility, especially at acidic pH, has often been utilized as a supplement for fruit juice and acidic drinks to enhance their nutritional quality. In addition, protein hydrolysates could be used as a supplementary diet for people with special needs such as elderly people and sportsmen, and for weight control.

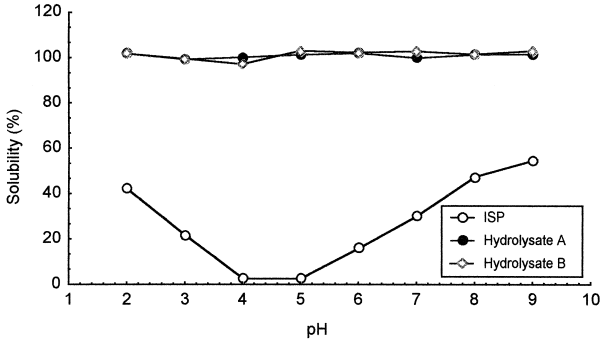

Fig. 4. Effect of pH (2–9) on solublity of intact ISP and its hydrolysates. Results are mean values of at least three measurements. Standard deviations did not exceed 2.5% of the recorded values.

3.3.2. Water sorption

Fig. 5 shows water sorption isotherms for intact ISP and its hydrolysates at 22°C. Water sorption of hydrolysates A and B increased 1.8–2.9 times and 2.1–3.4 times at water activity of 0.6–0.9 as compared to intact ISP, respectively. This suggests that the hydrolysates were more effective water binding agents than the intact ISP. Isolated soy protein contains a relatively large amount of ionizable polar amino acids such as glutamic and aspartic acids (Table 1) which can bind almost 3 times as much water as that of non-ionizable polar groups (Chou & Morr, 1979; Deeslie & Cheryan, 1988). In the native protein, some of these groups are buried and thus are not available for water binding. The results also suggest that the hydrolysates could be used as an additive in intermediate-moisture (IM) foods to bind water and improve the texture. Cheryan and Deeslie (1984) patented protein hydrolysates produced by ultrafiltration membranes and found that they were particularly effective for increasing nutrition and improving texture in various IM foods, such as high energy bars, moist-packaged animal foods and space foods.

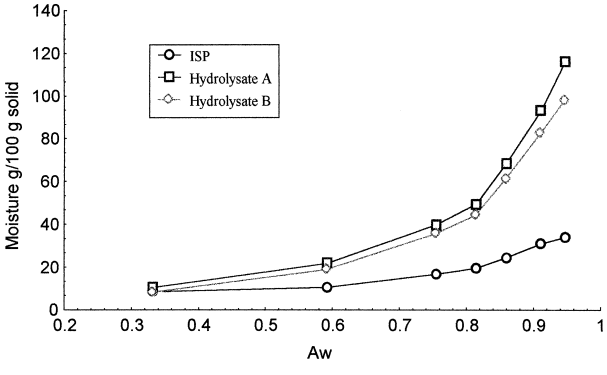

Fig. 5. Water sorption isotherms of ISP and its hydrolysates at 22°C. Results are mean values of at least three measurements. Standard deviations did not exceed 2.5% of the recorded values.

3.3.3. Antioxidant activity

Table 3 illustrates the antioxidant activities of ISP and its hydrolysates in soybean oil in terms of oxidative stability index (OSI). A higher OSI value represents a greater antioxidant activity in the soybean oil system. The results indicate that the antioxidant activity of ISP could be enhanced by enzyme hydrolysis of ISP. Both hydrolysates (A and B) gradually increased their antioxidant activity as well as oxidative stability with increase of their concentrations. Hydrolysate B from the 30,000 daltons MWCO membrane showed a much higher OSI value than hydrolysate A from the 3000 daltons MWCO membrane. At a concentration of 5%, oxidative stability of hydrolysates B and A increased 71.9 and 43.8% as compared with control, respectively. According to Chen, Muramots, and Yamauchi, (1995), antioxidative peptides from hydrolysates of soybean β-conglycinin are composed of 5–16 amino acid residues with molecular weights between 500 and 2000 daltons. They concluded that the antioxidant activity of hydrolysates was an inherent characteristic of amino acid sequences of peptides which depended on the specificity of the protease. Table 2 shows that the peptide content of hydrolysate B has 7% more of the 500-2000 dalton fraction than hydrolysate A. This may be why hydrolysate

Table 3
Effect of the concentration of ISP and its hydrolysates on their antioxidative activity in terms of oxidative stability index (OSI) at 120°C

	OSI	Percent increase in oxidative stability
Control	3.13 ± 0.07^{a}	
Hydrolysate A		
0.1% (w/w)	3.13 ± 0.07^{a}	0
0.5%	3.26 ± 0.04^{a}	3.1
1.0%	3.36 ± 0.14^{b}	9.4
2.5%	4.07 ± 0.04^{d}	28.1
5.0%	4.57 ± 0.03^{f}	43.8
Hydrolysate B		
0.1%	3.08 ± 0.07^{a}	0
0.5%	3.26 ± 0.11^{ab}	6.3
1.0%	3.63 ± 0.05^{c}	15.6
2.5%	4.23 ± 0.10^{e}	36.7
5.0%	5.36 ± 0.11^{g}	71.9
ISP		
0.1%	3.03 ± 0.06^{a}	0
0.5%	2.98 ± 0.15^{a}	0
1.0%	3.05 ± 0.11^{a}	0
5.0%	3.39 ± 0.04^{b}	7.0
0.1%BHA	3.18 ± 0.07^{ab}	1.6
0.1%BHT	4.44 ± 0.04^{a}	39.8

Results are mean values of three measurements ± standard deviation. Values in each row with the same superscript are not significantly different ($p > 0.05$) from one another. % increase in oxidative stability = 100×(experimental OSI−Control OSI)/ Control OSI. Control = without antioxidant.

B has a higher antioxidant activity than hydrolysate A. Several studies of protein hydrolysates have reported antioxidant activity (Bishov, Naits, Yakoo, & Fujumaki Henick, 1975; Yamaguchi et al., 1980; Yee, Shipe, & Kinesells 1980; Shahildi & Amarowicz, 1996). The results suggest the antioxidant activity of protein hydrolysates is affected by their composition of amino acids and peptides which were mainly governed by enzyme hydrolysis and the selected MWCO membrane.

4. Conclusion

The continuous HF membrane reactor proved to be a useful means to produce a protein hydrolysate with specific functional properties.

The hydrolysates with a wide range of pH solubility have the potential to be used as nutritional supplements in clear acidic beverages, and with a larger water-binding capacity can improve the texture and shelf-stablity of intermediate-moisture food. In addition, the hydrolysates with antioxidant activity may increase the stability of lipids and lipid-containing foods.

Acknowledgement

The authors would like to thank the Department of Industrial Technology in the Ministry of Economic Affairs, Republic of China, for financial support under grant no. 87-EC-2-A-17-0237.

References

AOCS (1989). *Official Methods and Recommended Practices of the American Oil Chemists' Society* (4th ed.). Champaign, IL: AOCS.

Bishov, S. J., & Henick, A. S. (1975). Antioxidant effect of protein hydrolyzates in freeze-dries model systems: synergistic action with a series of phenolic antioxidants. *Journal of Food Science, 40*, 345–348.

Bouhalab, S., Molle, D., & Lenil, J. (1993). Continuous hydrolysis of β-casein in a membrane reactor: preparation of a bioactive peptide. *Biotechnology Letters, 15*, 609–702.

Chen, H. M., Muramoto, K., & Yamauchi, F. (1995). Structural analysis of antioxidative peptides from soybean. *Journal of Agricultural and Food Chemistry, 43*, 574–578.

Cheryan, M., & Deeslie, W. D. (1983). Soy protein hydrolysis in membrane reactors. *Journal of the American Oil Chemists' Society, 60*, 1112–1115.

Cheryan, M., & Deeslie, W. D. (1984). *Protein hydrolysate*. US Patent 4,443,540.

Chiang, W. D., Cordle, C. T., & Thomas, R. L. (1995). Casein Hydrolysate produced using a formed-in-place membrane reactor. *Journal of Food Science, 60*, 1349–1352.

Chou, D. H., & Morr, C. V. (1979). Protein-water interactions and functional properties. *Journal of the American Oil Chemists' Society, 56*(1), 53A–62A.

Deeslie, W. D., & Cheryan, M. (1981). Continuous enzymatic modification of proteins in an ultrafiltration reactor. *Journal of Food Science, 46*, 1035–1042.

Deeslie, W. D., & Cheryan, M. (1988). Functional properties of soy protein hydrolysates from a continuous ultrafiltration Reactor. *Journal of Agricultural and Food Chemistry, 36*, 26–31.

Frokjaer, S. (1994). Use of hydrolysates for protein supplementation. *Journal of Food Science, 59*, 86–88.

Iacobucci, G. A., Myers, M. J., Emi, S., & Myers, D. V. (1974). Large scale continuous production of soybern protein hydrolysate in a constant flux membrane reactor. *Proc. IV Int. Congr. Food Sci. Technol.*, Madrid. Instituto de Agroquimicay Tecnologia de Alimentos, Valencia. (Vol. V, pp. 83–95).

Lang, K. W., McCune, T. D., & Steinberg, M. P. (1981). A proximity equilibrium cell for determination of sorption isotherm. *Journal of Food Science, 46*, 936–938.

Lin, S. B., Chiang, W. D., Cordle, C. T., & Thomas, R. L. (1997). Functional and immunological properties of casein hydrolysate produces from a two-stage membrane system. *Journal of Food Science, 62*, 480–483.

Mahmoud, M. I., Malone, W. T., & Cordle, C. T. (1992). Enzymatic hydrolysis of casein: Effect of degree of hydrolysis on antigenicity and physical properties. *Journal of Food Science, 57*, 1223–1229.

Mahmoud, M. I. (1994). Physicochemical and functional properties of protein hydrolysates in nutritional products. *Journal of Food Science, 59*, 89–95.

Mannhein, A., & Cheryan, M. (1990). Continuous hydrolysis of milk protein in a membrane reactor. *Journal of Food Science, 55*(390), 381–385.

Phillips, R. D., & Beuchat, L. R. (1981). Enzyme modification of proteins. Chpt. 13 In J. P. Cherry (Ed.), *Protein Functionality in Foods* (pp. 275–298). Symposium Series 147. Washington, DC: American Chemical Society.

Shahildi, F., & Amarowicz, R. (1996). Antioxidant activity of protein hydrolyzates from aquatic species. *Journal of the American Oil Chemists' Society, 73*, 1197–1199.

Simpson, R. J., Neuberger, M. R., & Liu, T. Y. (1976). Complete amino acid analysis of protein from a single hydrolysate. *Journal of Biological Chemistry, 251*, 1936–1940.

Sims, K. A., & Cheryan, M. (1992). Continuous production of glucose syrup in an ultrafiltration reactor. *Journal of Food Science, 57*, 163–166.

Turgeon, S. L., Gauthier, S. F., & Paquin, P. (1992). Emulsifying property of whey peptide fractions as a function of pH and ionic strength. *Journal of Food Science, 57*, 601–604, 634.

Xavier, A. M. R. B., Goncalves, L. M. D., Moreira, J. L., & Carrondo, M. J. T. (1995). Operational patterns affecting lactic acid production in ultrafiltration cell recycle bioreactor. *Biotechnology and Bioengineering, 45*, 320–327.

Yamaguchi, N., Naito, S., Yokoo, Y., & Fujimaki, M. (1980). Oxidative stability of dried model food consisted of soybean protein hydrolyzate and lard. *Nippon Shokuhin Kogyo Gakkaishi, 27*, 51–55.

Yee, J. J., Shipe, W. F., & Kinesella, J. E. (1980). Antioxidant effects of soy protein hydrolysates on copper-catalyzed methyl oxidation. *Journal of Food Science, 45*, 1082–1083.