Technical

Preparation of Low-Phytate Rapeseed Protein by Ultrafiltration: II. Membrane Development and Testing

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ABSTRACT

The feasibility of using ultrafiltration (UF) to eliminate phytate from aqueous sodium chloride extracts of rapeseed was studied. With a proper choice of casting solution composition and casting conditions, high flux asymmetric cellulose acetate membranes of different surface porosity capable of giving different levels of phytate-protein separation were effectively created. The efficiency of the cast cellulose acetate membranes on phytate-protein separation was greatly influenced by the solution pH. The presence of sodium chloride in the processed solution was important to obtain a low retention of phytate. Complete removal of phytate from rapeseed extract with little or no loss of proteins was achieved using continuous diafiltration.

INTRODUCTION

In our previous paper (1), it was shown that complete removal of phytate from Tower and Candle variety rapeseed meal and flour can be achieved by extraction using aqueous sodium chloride solution. Substantial amounts of proteins occur along with phytate and other phosphorus-containing substances in the extract liquid. The recovery of these proteins as nutritionally high quality isolates can be achieved by precipitation (2). However, during protein precipitation, phytate reacts with the proteins to form insoluble protein-phytate complexes which become concentrated together with the protein in the isolate product.

Since the development of the first asymmetric cellulose acetate membrane by Loeb and Sourirajan (3), ultrafiltration (UF) and reverse osmosis (RO) membrane systems have been used in processing wheys for product recovery and pollution abatement. Okuba et al. (4), Hartman (5), de Rham and Jost (6), and Omosaiye and Cheryan (7) have reported studies that involved the use of ultrafiltration to prepare low-phytate protein from soybean extract. Membranes of appropriate porosity and characteristics can be fabricated for protein fractionation, purification or concentration. This paper deals with the development of suitable cellulose acetate membranes to produce low-phytate protein from rapeseed extract.

MATERIALS AND METHODS

Preparation of Rapeseed Extract

The rapeseed extract was prepared by placing 6.0 g of the deoiled, dehulled Candle flour and 3 L of 4% (w/v) aqueous sodium chloride solution in a stoppered 6-L extraction flask which was mechanically mixed with a magnetic stirrer for 3 hr. The insoluble material was removed by vacuum filtration using Whatman 114 filter paper. The extraction was conducted at 24 ± 2 C. The pH of the extracted liquid phase was adjusted with reagent-grade hydrochloric acid and sodium hydroxide when applicable. The analysis of the total nitrogen, total phosphorus and phytate phosphorus of the air-dried Candle flour was (wt %) 6.66, 1.40 and 1.16, respectively. Kjeldahl nitrogen content was determined according to Lang (8) whereas the total phosphorus and phytate phosphorus were determined according to Pons et al. (9) with some modifications made by the authors. Protein content was determined by the Coomassie Brilliant Dye binding method of Bradford (10).

Membrane Casting Procedure

Flat, high flux, asymmetric cellulose acetate ultrafiltration membranes were made in the laboratory by hand casting on glass plates. A casting solution of cellulose acetate, acetone, water and magnesium perchlorate was used. After casting, the membranes were first immersed in an ethanol gelation bath to control porosity (11) and then in a water bath for washing and to ensure complete gelation. The finished membrane sheets were then cut to size and stored in 20% (v/v) ethanol solution at 4 C. The thickness of the cast membrane was ca. 0.4 mm. The composition (wt %) of the membrane casting solution (12) and the membrane casting conditions were as shown in Table I.

Description of Apparatus and Operation

The design of the ultrafiltration cell unit used in this investigation was obtained from the National Research Council of Canada (Ottawa) Chemical Engineering group of the Division of Chemistry. The cell was similar to that reported by Sourirajan (13), except that the flow pattern was modified to increase fluid velocity and minimize concentration polarization.

A schematic flow diagram of the UF system is presented in Figure 1. The system was simple and easy to operate. A

TABLE I

Membrane Casting Details

A. *Composition of the membrane casting solution*	(wt %)
Cellulose acetate (Eastman grade 400-25)	= 14.8
Acetone	= 63.0
Deionized distilled water	= 19.9
Magnesium perchlorate	= 2.3
B. *Membrane casting condition*	
Temperature of the casting solution	= 4 ± 1 C
Temperature of the casting atmosphere	= 22 ± 2 C
Solvent evaporation time	= 1 min
Temperature of the ethanol gelation bath	= 22 ± 2 C
Gelation time	= 15 min
Temperature of the leaching bath	= 22 ± 2 C
Leaching time	= 1 hr

high capacity Pulsafeeder PULSA 7120 diaphragm-metering pump was used to pump the feed solution under pressure through a series of 6 cells. (Note that only one cell unit is shown in the diagram.) A Pulsatrol pulsation dampener pressurized with nitrogen gas was employed to minimize the pressure fluctuations in the cell units. The needle valve downstream regulated the operating pressure. The operating pressure was measured with pressure gauges located before and after the cell units. Six cells were used in series to permit 6 membranes to be tested simultaneously. The entire system was constructed from 316 stainless steel.

All experiments on the UF system were conducted at a laboratory temperature of 24 ± 2 C. The feed solution flow rate was 2.5 L/min and the operating pressure was 137.9 KPa. The membranes were pretreated in the apparatus with pure water at a pressure of 344.7 KPa for 30 min in order to stabilize their porous structure. Except for the continuous diafiltration, the permeate samples were collected at the steady state condition, generally after 45 min of total reflux operation. The reported permeation rates are those adjusted to 25 C using the relative viscosity and density data for pure water. The solute separation was defined as:

$$\% \text{ solute retention} = 1 - \frac{\text{concentration of permeate (ppm)}}{\text{concentration of feed (ppm)}} \times 100$$

$$\% \text{ solute removal} = 1 - \frac{\text{concentration of final bulk solution (ppm)}}{\text{concentration of initial bulk solution (ppm)}} \times 100$$

RESULTS AND DISCUSSION

The initial criterion that determines the subsequent performance of a membrane is the composition of the casting solution. Based on the correlation work reported by Kutowy and Sourirajan on 23 different casting solution compositions of cellulose acetate, acetone and aqueous magnesium perchlorate, the membrane casting solution composition used is particularly suitable for making high-flux UF membranes (14). Furthermore, the use of aliphatic alcohols in the gelation bath promotes the development of higher flux cellulose acetate membranes (15). In view of this, effects of the amount of ethanol in the gelation medium on membrane performance were investigated.

By varying the ethanol-water composition of the gelation medium, membranes of different degrees of porosity and pore size were obtained as demonstrated in Figure 2. The pH of the feed solution was 6.3 and was not adjusted. From the graph, it was observed that membranes made using a gelation bath whose composition ranged from 38.00 to 47.50% (v/v) ethanol were most suitable for this particular rapeseed protein-phytate separation. These membranes were characterized by high protein retention and a corresponding low phytate retention as indicated by their percentage retention of total nitrogen and total phosphorus, respectively. Their relatively high product permeation rates were also quite attractive.

Preliminary work on the effects of pH on the feed solution on performance of the membranes was very encouraging. Figure 3 is a plot of data obtained for cellulose acetate membranes Et-38.42 and Et-41.80. The code designates the gelation bath composition as (v/v %) ethanol. Protein-phytate separation was greatly improved by shifting the operating pH from the normal 6.3 to 5.5. A much higher protein retention, and a correspondingly much lower phytate retention, were obtained for both membranes. Moreover, protein retention for membrane Et-38.42 approached 100% at a pH of 5.5, which corresponded to no loss of protein. Whether effects of pH can be attributed to the "apparent" bulk isoelectric point of the protein mixture is yet to be investigated. A more thorough and more conclusive investigation on influence of solution condition and operating parameters will be reported in the near future.

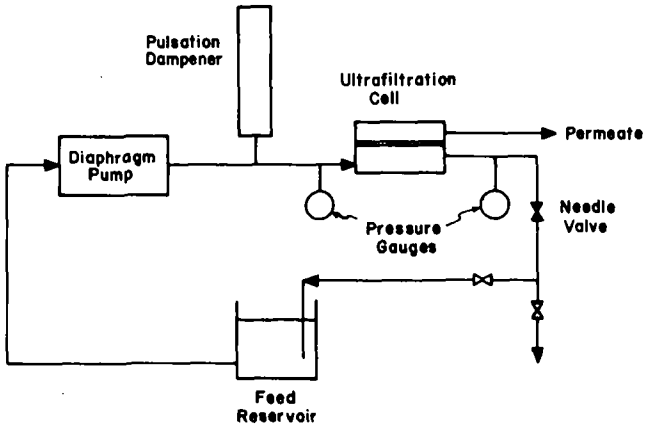

FIG. 1. Schematic flow diagram of the UF system.

Prior to the determination of a most favorable membrane operating condition for separation of phytate from the dissolved proteins, it is important to investigate beforehand whether complete removal of phytate from dissolved proteins is attainable by membrane processing. This was done by continuous diafiltration using the previously described UF system. During the operation, the total bulk feed solution volume was kept constant at 2.5 L by continually adding fresh 4% (w/v) aqueous solution of sodium chloride to the feed tank at a rate equal to that of the total permeate from the 6 cell units. Figure 4 displays data obtained using membrane Et-39.9. This figure shows that

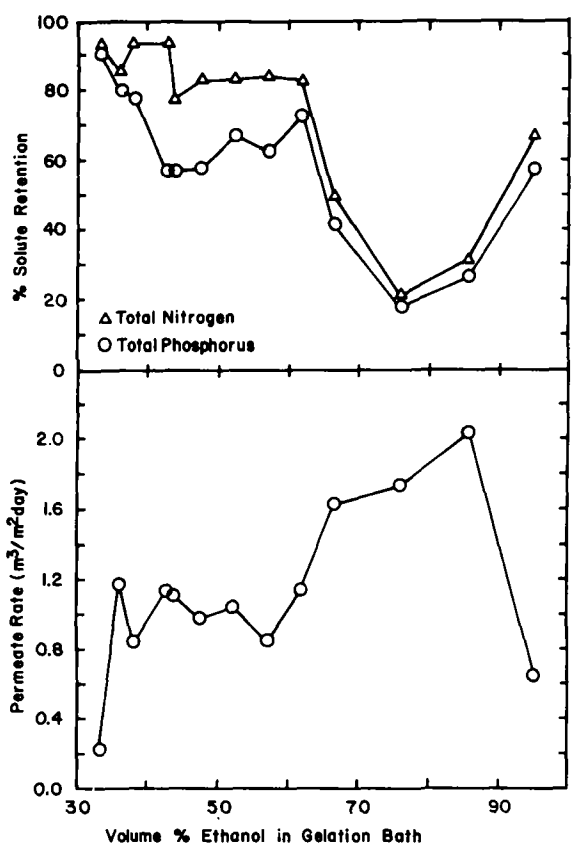

FIG. 2. The effect of gelation bath composition on membrane retention and permeate rate.

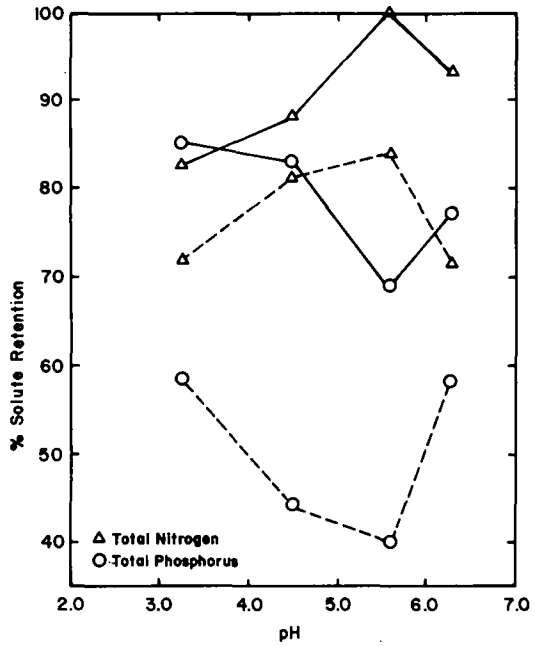

FIG. 3. The effect of pH on membrane solute retention. —— Membrane Et-41.80; ——— membrane Et-38.42.

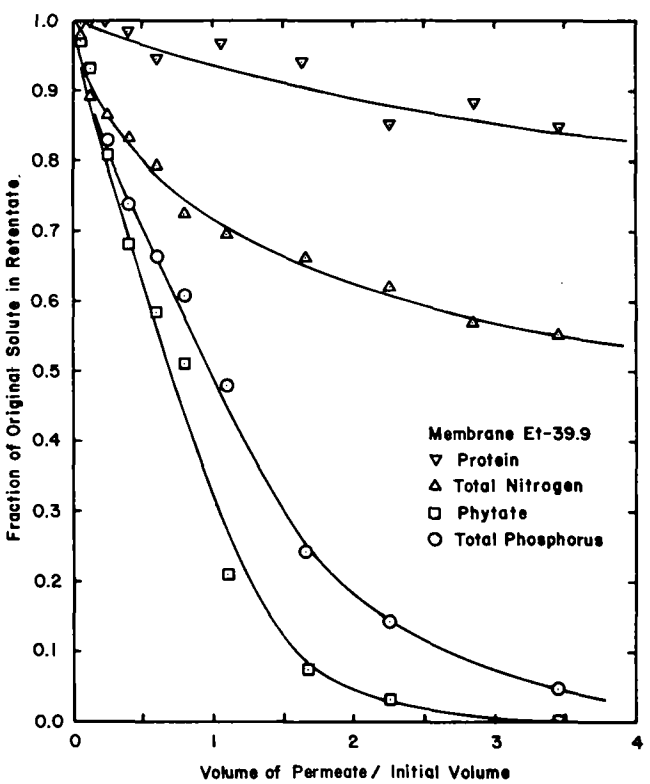

FIG. 4. Separation of solutes by continuous diafiltration.

complete removal of phytate is attainable. At a total permeate volume of 5 L, which is twice the volume of the total bulk solution, the corresponding percentage removal of total phosphorus, phytate, total nitrogen and protein was 85.38, 95.57, 39.89 and 8.59, respectively. It is observed from Figure 4 that, after a total permeate volume of 1.5 times the volume of the total bulk solution, the total N curve is in parallel with the protein curve. Prior to this, the greater slope of the initial portion of the total nitrogen curve can be attributed to removal of the smaller nonprotein nitrogeneous compounds. Loss of protein is relatively low at 8.59%, and can be minimized by the use of higher protein rejection membranes. Furthermore, operating under a more favorable operating condition, such as pH 5.5, will improve membrane performance both in the amount of protein recovery and the rate of phytate removal.

The sodium chloride in the processed solution must be maintained at a certain concentration during continuous diafiltration to keep the protein in solution and to retain the phytate-protein selectivity of the membrane. This was observed when experimental runs using water extracts of the rapeseed samples failed to produce a reasonable protein-phytate separation. Further experiments, using only sodium phytate in water and the same membranes, revealed that phytate retention of the cellulose acetate membrane was greatly influenced by the presence of sodium chloride. For example, retention of phytate was measured to be about 15% in the presence of sodium chloride, and 90% in the absence of sodium chloride. This effect is being investigated further in our laboratory.

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