ULTRAFILTRATION OF FISH PROCESSING WASTES USING POLYAMIDE MEMBRANES

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ABSTRACT

Polyamide membranes were prepared by dissolving nylon-6 fibers in a mixture of hydrochloric acid, ethanol and water, spreading the dope into a thin film and film hardening in a water bath. Water flux through the membrane decreased linearly with membrane thickness. Membrane storage under water caused reduction in total membrane pore area and in the number of tubular passages as shown by scanning electron microscopy.

Ultrafiltration of waste tuna and sardine broths using freshly prepared membranes showed a molecular cut-off of 10-30 kilodaltons for protein in the permeate. The concentration factor (ratio of solute concentration in the retentate relative to the fresh sample) was in the range of 1.8-3.5 for both samples. The rejection coefficient σ was 50.2-84.0 for protein and 40.8-73.0 for carbohydrate.

Introduction

Ultrafiltration (UF) has been applied extensively to the utilization of numerous food processing wastes such as those from the dairy (Marshall, 1982; Muller, 1983) and fruit juice industries (Hasting *et al.*, 1983; Heatherbell *et al.*, 1983). Unfortunately, UF applications in utilizing fish canning wastes seem to be limited.

Tuna and sardine canning has become an important foreign exchange-earning industry in the ASEAN region. An average sized fish canning factory in the Philippines, for example, processes more than 100 tons of fish daily during the peak season; more than 50% of this weight ends up as waste. The factory produces two important liquid wastes, namely tuna and sardine broths which are produced daily in volumes of approximately 6 and 30 metric tons, respectively. These waste fish broths contain more than 2% total solids, mainly protein. Protein recovery from the broths would result in substantial reduction in biochemical oxygen demand of the effluent as well as availability of a valuable nutrient for food.

The present paper describes the preparation of polyamide (nylon-6) membranes and their use in the ultrafiltration of waste tuna and sardine broths. The membrane and fish broths were also characterized in order to interpret the ultrafiltration data.

Materials and Methods

Preparation and selection of polyamide membranes

Ultrafiltration (UF) membranes were made from nylon-6 using the procedure of Susanto *et al.* (1982). Approximately ten grams of nylon-6 fibers were dissolved at room temperature (27-30°C) in a solvent mixture containing 8.0 ml distilled water, 11.0 ml concentrated hydrochloric acid and 1.0 ml ethanol. This mixture or dope was stirred for 15 minutes at 500 rpm with a GKH stirrer type HST 220N connected to a Series H motor controller.

The homogenized dope was spread evenly with a cylindrical glass rod over a flat horizontal glass plate (20 cm x 20 cm). The plate had two layers of transparent plastic tape near the edges for adjusting the dope film thickness. The dope-coated glass plate was then quickly immersed in distilled water and the dope film was allowed to harden for about a minute.

The resulting polyamide sheet was detached from the glass plate and visually inspected. Areas apparently free of pinholes and of uniform thickness were cut into 76 mm diameter discs. The latter were tested for uniformity of thickness using a spring-activated thickness gauge (Mitutoyo Model 7321). The thickness was measured at the center and at four equally spaced points near the edge of each membrane. Discs were discarded if their thickness variation from the average value, based on measurements at the five points, was more than 10%.

Measurements of membrane water flux and molecular weight cut-off

Water flux was measured for the selected membranes at 300 kPa (45 psig) pressure. A Thermolyne magnetic stirrer (Model S-7225) was used in all UF runs at about 500 rpm.

Ultrafiltration of waste fish broths and standard dextran solutions was done in an stirred-cell Amicon apparatus (Model 401S) at 300 kPa and 500 rpm stirrer speed. Initial sample volumes were uniformly set at 100 ml; about 70 ml of permeate were collected for each UF run.

The molecular cut-off of the membranes was determined using standard dextrans (Sigma Chemical Co., St. Louis, Mo., U.S.A.) with molecular weights (MW) of 153, 249, 500 and 5,000-40,000 kilodaltons. About 100 ml of the dextran solution at a concentration of 200 μg/ml, were ultrafiltered and 10 ml fractions (total = 70 ml) were collected. The retentate and permeate samples were analyzed for carbohydrate using the phenol-sulfuric acid method of Dubois *et al.*, (1956).

Scanning electron microscopy (SEM)

The UF membranes were thoroughly air dried and then cut into strips about 2.5 mm wide and 8-12 mm long. The ends of the membrane strips were glued on to the top of the sample discs such that each strip was twisted and partially folded in order to allow examination of the membrane's top and bottom surfaces and its cross-section. Electrically conductive silver paste was used for pasting four membrane strips to each sample disc. These were then coated for five minutes with a mixture of gold and palladium in a Hitachi E101 ion sputter. A Hitachi S-510 scanning electron microscope was used for examining the membrane strips. The acceleration voltage of the electron beam was usually set at 15 kilovolts.

Analysis and ultrafiltration of waste fish broths

Samples of waste tuna and sardine broths were obtained from a fish canning factory (Century Canning Corp., Taguig, Metro Manila). Determination of protein and carbohydrate concentrations was based on the methods of Lowry *et al.* (1951) and Dubois *et al.* (1956), respectively. The fish broth samples were dried at 105 ± 2°C to constant weight. The ash content of each broths dry matter was determined by heating to constant weight at 550°C.

Prior to gel chromatography, the tuna broth was extracted thrice, each time with three volumes of petroleum ether, and suspended particles in the defatted broth were removed by settling. The sardine broth, which was not defatted, was also freed of suspended matter. An aliquot (~ 0.2 ml) of each treated broth was introduced on top of a Bio-Rad econocolumn (1.0 cm diameter, 38 cm length and 40 cm pressure head) containing Bio-Gel A-0.5m (100-200μ) gel and eluted with 0.025 M sodium phosphate buffer, pH 7.0. A Gilson fractionator (Model FC-80K) was used for collecting 0.5 ml eluted fractions.

The following proteins were used as molecular weight standards: cytochrome C, 12.4 kD (kilodaltons); bovine pancreatic α-chymotrypsin, 25.31 kD; bovine serum albumin, 66 kD; and bovine liver β-D-galactosidase, 43 kD. Blue dextran (2 x 10^6D) at a concentration of 20 mg/ml, was used for measuring the column void volume. All protein and dextran standards were obtained from Sigma Chemical Co. The protein standards were mixed and dissolved in 5.0 ml buffer at a concentration of 2 mg/ml for each protein.

Ultrafiltration of the waste fish broths was done using the Amicon 401S stirred-cell assembly.

Results and Discussion

Two sets of the prepared polyamide membranes (20 and 24 membrane discs) which had a uniform thickness of 0.11 ± 0.01 mm and were free of pinholes were selected. Set I membranes, which had been stored under distilled water in the refrigerator for about four months, had a steady-state water flux of 98 ± 9 $L\text{-}m^{-2}\text{-}h^{-1}$

while set II membranes, which were freshly prepared, had a water flux of 168 ± 12 $L\text{-}m^2\text{-}h^{-1}$. The solute rejection coefficient σ for each membrane was calculated from the equation

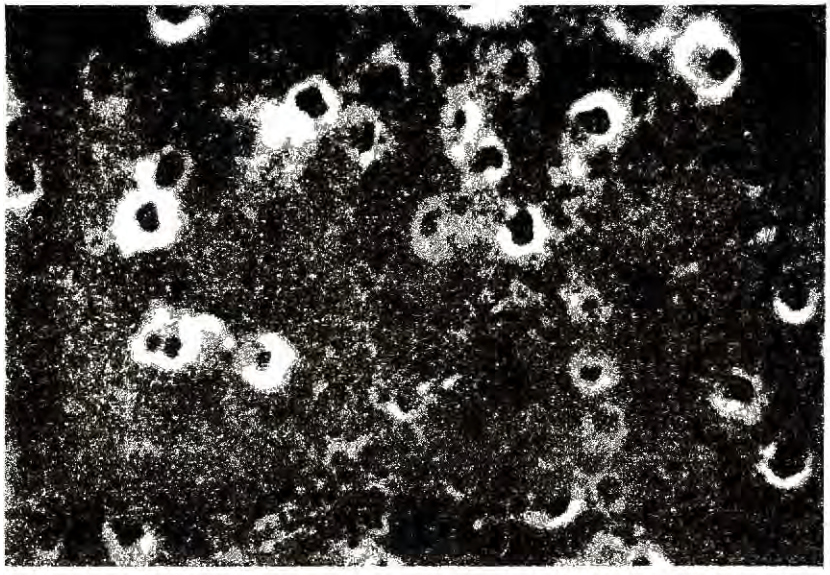
$$\sigma = 1 - (C_p/C_i) \quad (1)$$

where C_p and C_i are the solute concentrations in the permeate and bulk feed, respectively. Values of σ and the solute recovery, which was the solute mass ratio in the retentate and permeate relative to the initial (bulk feed sample), are presented in Table 1. The tabulated data indicate that the molecular weight (MW) cut-off for both membrane sets is close to 500 kD. Unfortunately, a more precise value could not be calculated due to the limited MW standards used.

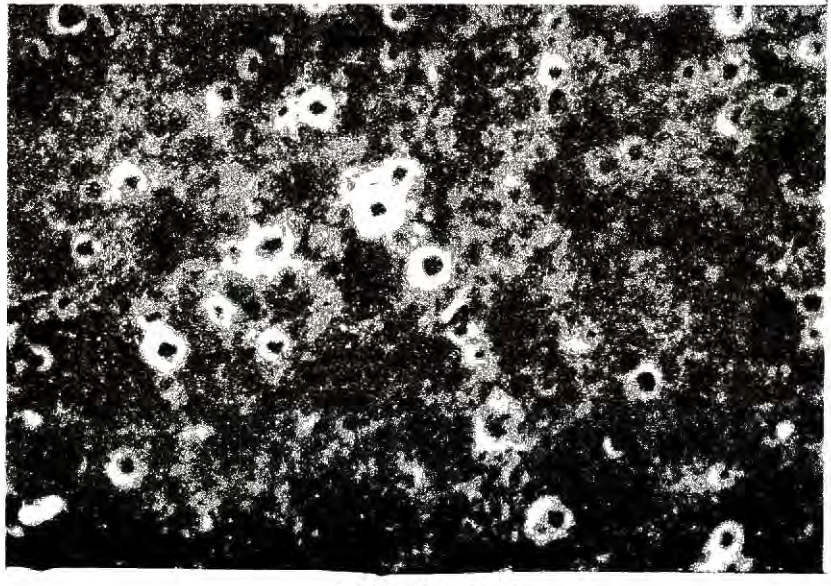
Table 1. Molecular cut-off properties of two sets of prepared polyamide membrane

Molecular weight of dextran (kD)	*Rejection cofficient σ (%)*		*Solute recovery (%)*	
	Set I	*Set II*	*Set I*	*Set II*
153	6.2 ± 0.8	2.5 ± 0.2	95.6 ± 0.1	96.0 ± 0.7
249	7.1 ± 0.8	3.3 ± 0.3	93.3 ± 3.6	95.0 ± 0.4
500	96.2 ± 0.5	93.7 ± 0.8	108 ± 8	89.2 ± 0.1
$(5 - 40)\ 10^3$	96.8 ± 0.1	95.3 ± 0.3	84.2 ± 4.3	82.4 ± 0.4

The difference in water flux for the two sets of membranes may be explained in terms of the scanning electron micrographs (SEM), which are shown in Figs. 1-3, The top surface or (+) configuration of both membrane sets shows similar pore size and pore population (Fig. 1). However, the bottom membrane surface or (–) configuration (Fig. 2) was substantially different in appearance, with set II membrane having bigger pores. Measurement of the ratio of total pore area and membrane area (A_p/A_m) was done by cutting equal areas from photocopies of the SEM and getting the weights of the paper strips before and after burning out the pore sections using a red-hot nichrome wire (done in triplicate). The A_p/A_m values for set I and II membranes were 9.5 ± 0.9 and 20 ± 1%, respectively. Furthermore, the cross-sectional SEM (Fig. 3) shows a more open alveolar structure for set II membranes which would allow easier permeation of solvent through the membrane. The results indicate that the freshly prepared (set II) membranes are more porous,

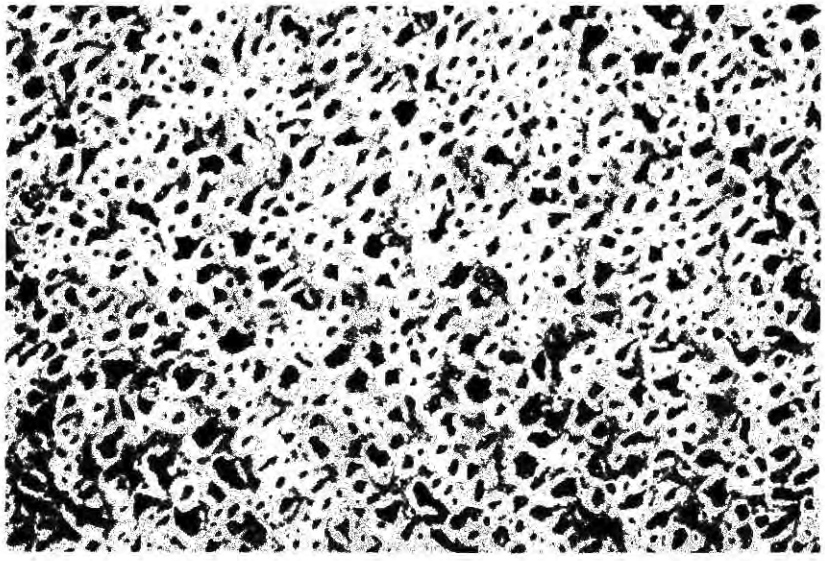


a.) Set I membranes

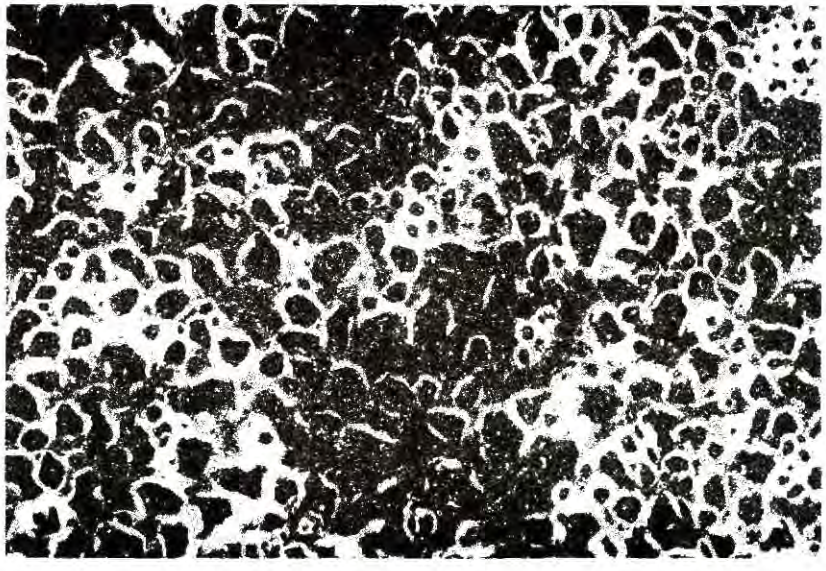


b.) Set II membranes

Fig. 1. Top surface view of the polyamide membranes.



a.) Set I membranes



b.) Set II membranes

Fig. 2. Bottom surface views of the polyamide membranes.

a.) Set I membranes

b.) Set II membranes

Fig. 3. Cross section of the polyamide membranes.

resulting in greater water flux; storage in water for several months apparently led to membrane shrinkage and reduction in water flux.

The linear inverse relationship between water flux and membrane thickness for freshly prepared membranes is presented in Fig. 4; the negative correlation coefficient was found to be greater than 0.99. This observation is expected for "tapered-pore" microporous membranes (Henry, 1980), such as that used in the present study, based on solvent flux equations for ultrafiltration (Fane, 1980; 1985).

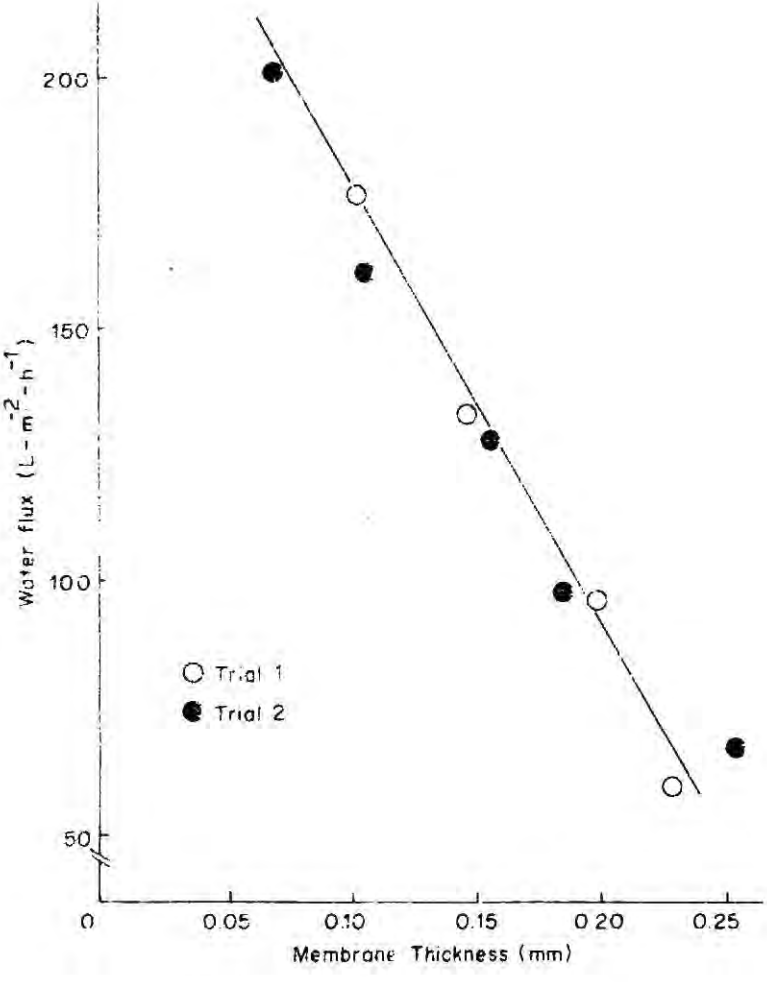


Fig. 4. Relationship between waterflux and thickness of polyamide membrane

The molecular cut-off properties of the prepared membranes are given in Table 1 while the fouling effects of dextran molecules of varying molecular weight (MW) on the prepared (set I) membranes are shown in Fig. 5. A greater reduction in water flux was observed after ultrafiltration of the bigger dextran molecules. However, prior ultrafiltration of bovine serum albumin (BSA) did not significantly alter the fouling effects of dextran. The latter probably clogged the membrane pores and filled up alveolar spaces, thereby hindering water permeation. Interestingly, a relatively high rejection coefficient, σ of approximately 60% was observed for BSA (MW = 66 kD); corresponding σ values for 249 kD and 500 kD dextran were 8.0 and 97.2%, respectively. Concentration polarization could be invoked in order to explain this observation based on the fact that the BSA concentration used was about five times that of the dextran. Another possibility is that the molecular weight calibration of the membranes using dextran molecules is not valid when applied to permeating protein molecules; instead protein MW should be used. This matter is discussed later in the paper in relation to ultrafiltration of the protein and carbohydrate solutes of fish broths.

Some proximate data for waste tuna and sardine broths are given in Table 2. Tuna broth is the liquid obtained after steaming fresh whole tuna prior to separation of fish meat for canning. On the other hand, sardine broth is obtained by boiling eviscerated sardines in tap water; the water is drained off (as sardine broth)

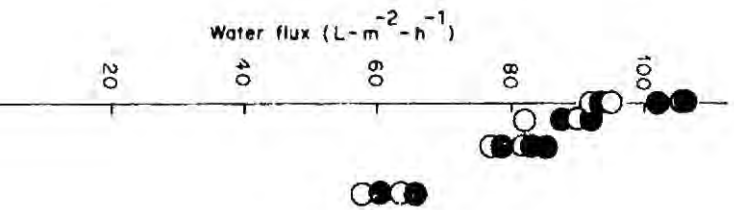
Table 2. Proximate analysis of tuna and sardine broths

Component	*Tuna broth*	*Sardine broth*
Total solids*	7.41 ± 0.74	2.38 ± 0.12
Protein**	35.1 ± 5.4	21.2 ± 1.5
Carbohydrate**	2.78 ± 0.09	1.11 ± 0.14
Fat*	0.347	0.218
Ash*	2.64 ± 0.06	0.390 ± 0.015

*percent by weight; **grams per liter of sample

and replaced with tomato sauce prior to canning. As shown in Table 2, tuna broth contains about three times more solids and much greater amounts of protein and ash compared to sardine broth. Protein is the predominant solute, especially for tuna broth. A small and similar amount of fat is found in both broths.

The results of gel chromatography (Pharmacia, 1974) of the waste fish broths through Bio-Gel A-0.5m are presented in Fig. 6 and 7. The top plot in each figure is the elution profile of the raw fish broth, while the bottom plot corresponds to the permeate after ultrafiltration through freshly-prepared polyamide membranes.


Water flux (L-m-2-h-1)
20
40
60
80
100

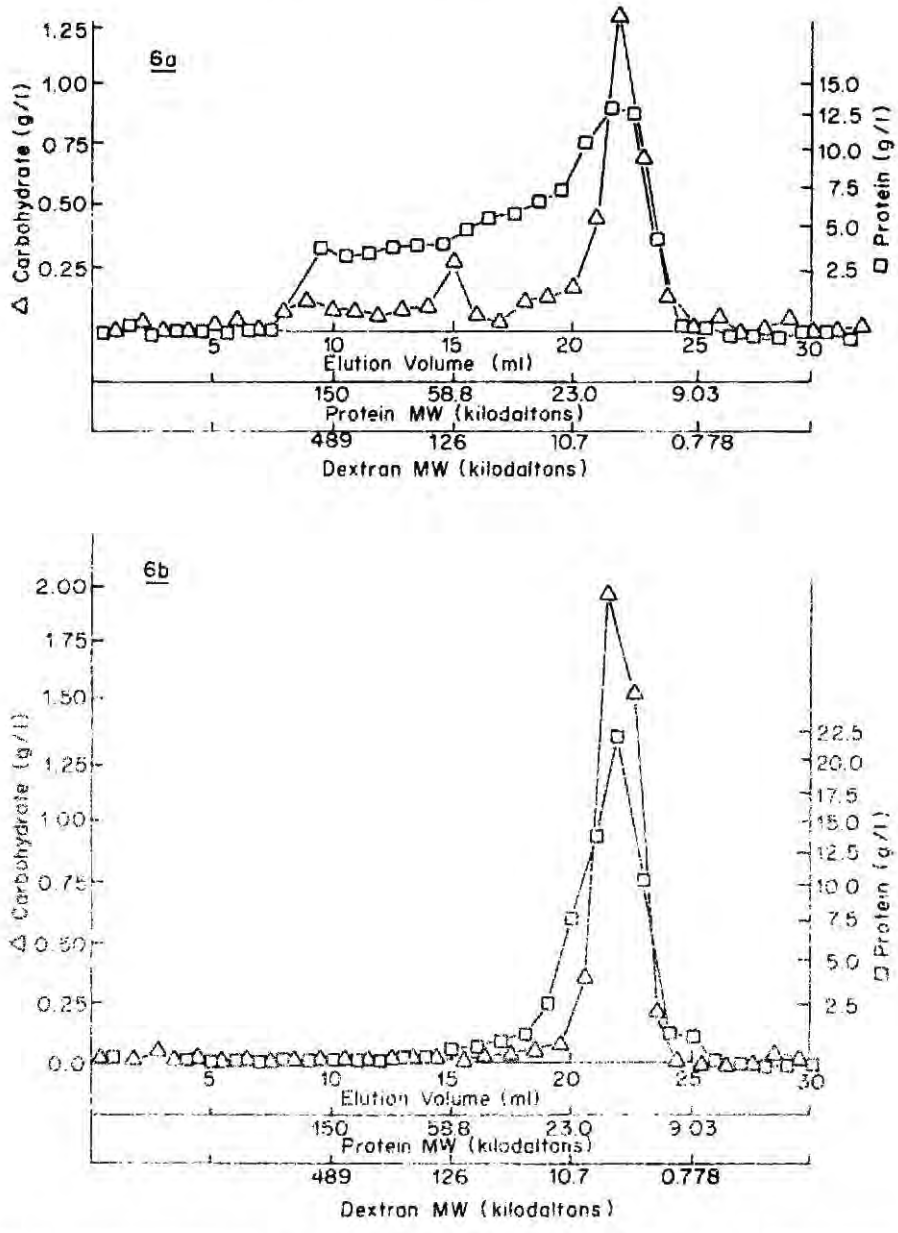


Fig. 6. Elution profiles of protein and carbohydrate in tuna broth sample (6a) and permeate (6b) through Bio-Gel A-0.5 column.

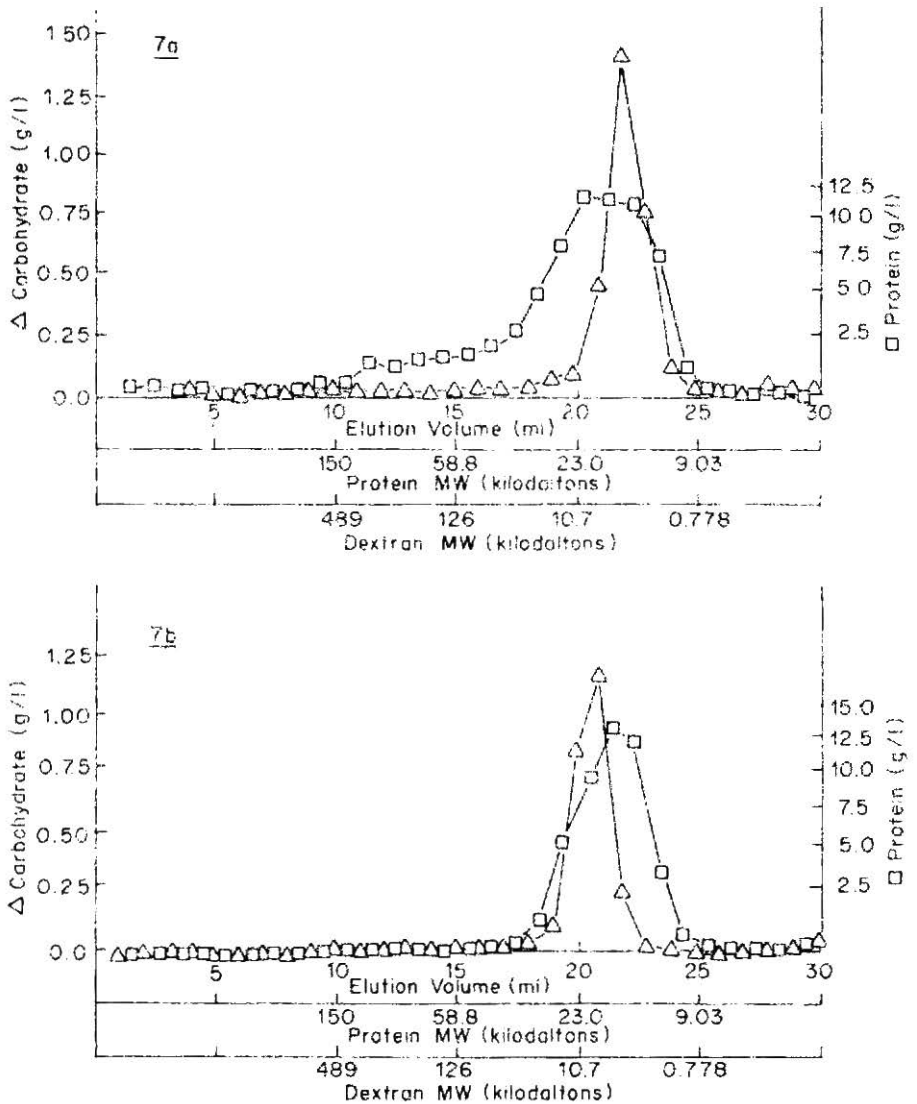


Fig. 7. Elution profiles of protein and carbohydrate in sardine broth sample (7a) and permeate (7b) through Bio-Gel A-0.5m column.

The horizontal scale (abscissa) is expressed in elution volume (ml) as well as in molecular weight (MW) of protein and dextran using available standards. It is interesting to note that the protein and carbohydrate elution peaks correspond to roughly the same elution volume, especially for the permeate. This could indicate that the carbohydrate is bound to the protein, e.g. as glycoprotein; an alternative, which is less probable, is that the carbohydrate and protein molecules are free but have roughly the same MW or dynamical size and shape. The absence of high MW (low-elution volume) solutes in the permeate, which are present in the raw samples, shows that these solutes were retained by the membrane. The MW of the protein molecule(s) in the permeate is in the range of 10-30 kD for both tuna and sardine broths. This low MW cut-off of the membrane relative to proteins supports the observation previously mentioned regarding the rejection coefficient of 60% for bovine serum albumin (MW = 66 kD). Therefore, the results indicate that the cut-off values for the membrane are quite different for protein and dextran molecules which imply the very different hydrodynamical properties of these molecules in relation to their molecular weights.

The summarized results of the ultrafiltration of tuna and sardine broths are given in Table 3. The results show that the temperature of membrane formation affects the ultrafiltration parameters; greater values of the solute rejection coefficient σ were obtained for membranes prepared at 27-30°C than at 4-6°C. However, slightly greater values of the concentration factor CF were obtained for the low-temperature membrane. Unfortunately, because of the large experimental variations in the results a meaningful analysis of the data is not warranted and only general conclusions can be made. The CF values for protein, which were in the range of 1.85-3.51 for both fish broths, as well as the rejection coefficient σ could probably be increased by improvement of the membranes. Thus, further research and development work is needed in order to make ultrafiltration a practical process for the concentration and utilization of waste fish broths.

Conclusions

Polyamide (nylon-6) ultrafiltration membranes were prepared using standard procedures reported in the literature. Water flux through the membrane was found to vary inversely in a linear manner with membrane thickness. Membrane storage under distilled water reduced water flux without significantly affecting molecular weight cut-off properties. Examination of the membranes by scanning electron microscopy (SEM) showed reduction in the ratio of pore area to membrane area (A_p/A_m) for the stored membranes as well as in the number of alveolar or tubular channels in the membranes.

Tuna and sardine broths were ultrafiltered using the freshly prepared polyamide membranes; the fresh broths and permeates were analyzed chemically and chromatographically using Bio-Gel A-0.5m column. The permeate elution profiles indicate a molecular weight cut-off of 10-30 kD for the protein solute of both

broths. The co-eluting protein and carbohydrate peaks in the permeate could mean the presence of glycoproteins in the broths. Values of the concentration factor were in the range of 1.85-3.5 for both samples. The rejection coefficients were in the range of 50.2-84.0 for protein and 40.8-73.0 for carbohydrate.

Table 3. Ultrafiltration parameters for waste tuna and sardine broths using membranes prepared at two sets of temperatures

Parameter	*Tuna broth*		*Sardine broth*	
	Protein	*Carbohydrate*	*Protein*	*Carbohydrate*
Membranes Prepared at 4 6°C				
Initial Conc.*	29.4 ± 3.6	2.87 ± 0.29	16.1 ± 0.4	1.74 ± 0.09
Retentate Conc.*	103 ± 16	6.84 ± 0.68	52.8	2.13
Permeate Conc.*	9.8 ± 1.8	1.48 ± 0.24	8.05	1.03
% Rejection	68.1 ± 2.2	48.6 ± 3.7	50.2 ± 1.0	40.8 ± 2.9
Conc. factor				
Experimental	3.51 ± 0.17	2.38 ± 0.03	3.27 ± 0.07	1.22 ± 0.06
Theoretical ≠	3.4	2.7	2.8	2.3
Membranes Prepared at 27-30°C				
Initial Conc.*	27.4 ± 1.3	2.63 ± 0.15	12.4 ± 1.1	1.29 ± 0.26
Retentate Conc.*	50.8 ± 0.1	4.57 ± 0.13	24.9 ± 0.2	1.71 ± 0.03
Permeate Conc.*	4.21 ± 0.29	0.68 ± 0.01	2.71 ± 0.12	0.41 ± 0.05
% Rejection	84.0 ± 0.7	73.0 ± 1.3	78.0 ± 0.9	62.4 ± 3.7
Conc. factor				
Experimental	1.85	1.74	2.02	1.32
Theoretical ≠	2.95	2.73	2.82	2.59

*Concentrations in g/L

≠ Calculated from the equation: $F = \sigma \frac{V_c}{V_r} + 1$

where the symbols are defined and related as follows:

F = concentration factor, σ = rejection coefficient (expressed as fraction), V_p = volume of permeate, V_r = volume of retentate, C_p = solute concentration in permeate, and C_r = solute concentration in retentate.

$$\sigma = 1 - \frac{C_p}{C_i} \; ; \; F = \frac{C_r}{C_i} \; ; \; C_r V_r + C_p V_p = C_i(V_r + V_p)$$

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Literatured Cited

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350-356.

Fane, A.G. 1980. Lecture notes on ultrafiltration (Part A). Seminar/Workshop Course on Membrane Technology and its Industrial Application – Workshop/Seminar Notes. January 29-31, 1980. Univ. of New South Wales, Sydney, Australia.

Fane, A.G. 1985. Membranes for downstream processing. Downstream Processing in Biotechnology – Course Notes. August 29-30, 1985. Univ. of New South Wales, Sydney, Australia.

Hasting, A.P.M., M. Cunningham, and R. Skeleton. 1983. Membrane application in apple juice processing. International Membrane Technology Conference – Abstracts of Papers, Univ. of New South Wales, Sydney, Australia, November 8-10, 1983. pp. 120-123.

Heatherbell, D.A., J.L. Short, B.R. Breslau, and B.A. Milnes. 1983. The application of ultrafiltration to fruit juice processing. International Membrane Technology Conference – Abstracts of Papers, Univ. of New South Wales, Sydney, Australia. November 8-10, 1983, p. 125.

Henry J.D. Jr. 1980. Reverse osmosis and ultrafiltration. Seminar/Workshop Course on Membrane Technology and its Industrial Application – Workshop/Seminar Notes. January 29-31, 1980. Univ. of New South Wales, Sydney, Australia.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the folin phenol reagent, *J. Biol. Chem.* 193: 265-275.

Marshall, S.C. 1982. Membrane technology in the Australian dairy industry. Proceedings – Second ASEAN Workshop on Membrane Technology, October 1-10, 1982, Bangkok, Thailand, pp. 250-266.

Muller, L.L. 1983. Recent applications of membrane in the dairy industry. International Membrane Technology Conference – Abstracts of Papers, Univ. of New South Wales, Sydney, Australia, pp. 178-180.

Pharmacia. 1974. Sephadex gel filtration in theory and practice (booklet) Pharmacia Fine Chemicals, Uppsala, Sweden.

Susanto, I.R., Djulia and A. Bakar. 1982. The effect of acid concentration and annealing on the properties of polyamide ultrafiltration membranes. Proceedings Second ASEAN Workshop on Membrane Technology, October 1-10, 1982, Bangkok, Thailand, pp. 46-50.