sulfonic acids according to well known pathways (Bauer and Cymerman, 1950). The necessary data to be collected before approval of any specific thiolsulfonate or thiosulfate as food additive could be obtained might include the identification and pharmacological evaluation of the chemical byproducts of the cooked flavor inhibition and of the products of decomposition of the additive itself.

The findings of the present work with whole milk might be applicable to evaporated skim milk and other heated dairy foods.

ACKNOWLEDGMENT

The author is indebted to Daniel L. Klayman, Division of Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, D. C., for helpful discussions.

LITERATURE CITED

Arnold, R. G., *J. Dairy Sci.* **52,** 1857 (1969).
Axelson, G., Hamrin, K., Fahlman, A., Nordling, C., Lindberg, B. J., *Spectrochim. Acta Part A* **23,** 2015 (1967).
Ballance, P. E., *J. Sci. Food Agr.* **12,** 532 (1961).
Bauer, L., Cymerman, J., *J. Chem. Soc.* 109 (1950).
Crawhall, J. C., Segal, S., *Nature (London)* **208,** 1320 (1965).
Demott, B. J., Gibbs, A. W., *J. Dairy Sci.* **49,** 1152 (1966).
Distler, H., *Angew. Chem. Int. Ed. Engl.* **6,** 544 (1967).
Ellis, G. P., *Advan. Carbohydr. Chem.* **14,** 63 (1959).
Emiliozzi, R., Pichat, L., *Bull. Soc. Chim. Fr.* 1888 (1959).
Eriksson, B., Rundfelt, M., *Acta Chem. Scand.* **22,** 562 (1968).
Ferretti, A., Flanagan, V. P., *J. Dairy Sci.* **54,** 1769 (1971).
Ferretti, A., Flanagan, V. P., *J. Agr. Food Chem.* **20,** 695 (1972).
Field, L., Owen, T. C., Crenshaw, R. R., Bryan, A. W., *J. Amer. Chem. Soc.* **83,** 4414 (1961).
Field, L., Ferretti, A., Crenshaw, R. R., Owen, T. C., *J. Med. Chem.* **7,** 39 (1964).
Gilman, H., Smith, S. E., Parker, H. H., *J. Amer. Chem. Soc.* **47,** 851 (1925).
Gruenwedel, D. W., Patnaik, R. K., *J. Agr. Food Chem.* **19,** 775 (1971).
Hartman, A. M., Dryden, L. P., in "Fundamentals of Dairy Chemistry," Webb, B. H., Johnson, A. H., Ed., AVI, Westport, Conn., 1965, pp 261–338.
Henry, K. M., Kon, S. K., Lea, C. H., White, J. C. D., *J. Dairy Res.* **15,** 292 (1948).
Hodge, J. E., *J. Agr. Food Chem.* **1,** 928 (1953).
Hodge, J. E., "Symposium on Foods. Chemistry and Physiology of Flavors," Schultz, H. W., Day, E. A., Libbey, L. M., Ed., AVI, Westport, Conn., 1967, pp 465–491.
Hutton, J. T., Patton, S., *J. Dairy Sci.* **35,** 699 (1952).
Josephson, D. V., *J. Agr. Food Chem.* **2,** 1182 (1954).
Keenan, T. W., Lindsay, R. C., *J. Dairy Sci.* **51,** 112 (1968).
Kiermeier, F., Hamed, M. G. E., *Z. Lebensm. Unters. Forsch.* **115,** 322 (1961).
Kobayashi, N., Fujimaki, M., *Agr. Biol. Chem.* **29,** 698 (1965).
Maillard, L. C., *C. R. Acad. Sci.* **154,** 66 (1912).
Mudd, S. H., Irreverre, F., Laster, L., *Science* **156,** 1599 (1967).
Patton, S., *J. Dairy Sci.* **38,** 457 (1955).
Patton, S., Josephson, D. V., *J. Dairy Sci.* **30,** 538 (1947).
Robinson, H. C., Pasternak, C. A., *Biochem. J.* **93,** 487 (1964).
Scanlan, R. A., Lindsay, R. C., Libbey, L. M., Day, E. A., *J. Dairy Sci.* **51,** 1001 (1968).
Schöberl, A., Wagner, A., in "Methoden der organischen Chemie (Houben-Weyl)," Müller, E., Ed., Vol. 9, G. Thieme-Verlag, Stuttgart, 1955, p 72.
Schönberg, A., Moubacher, R., *Chem. Rev.* **50,** 261 (1952).
Schutte, L., Koenders, E. B., *J. Agr. Food Chem.* **20,** 181 (1972).
Sörbo, B., *Acta Chem. Scand.* **12,** 1990 (1958).
Swan, J. M., *Nature (London)* **180,** 643 (1957).
Toennies, G., Lavine, T. F., *J. Biol. Chem.* **113,** 571 (1936).
Waley, S. G., *Biochem. J.* **71,** 132 (1959).

Received for review May 15, 1973. Accepted August 2, 1973. Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

# Preparation of Aqueous Beef Flavor Precursor Concentrate by Selective Ultrafiltration

Ahmed F. Mabrouk

Aqueous beef extract was fractionated by two ultrafiltration systems, batch-type and recirculating thin-channel, equipped with two membranes of different retentive capacities. Ultrafiltration was achieved in 2.5–10% of the time needed for exhaustive conventional dialysis. The influence of rate of stirring, pressure applied, and concentration of solute on ultrafiltration flux was studied in the batch system. Gel permeation chromatograms of beef flavor precursors prepared by conventional dialysis and ultrafiltration show a high degree of similarity beyond the void volume fraction. The three preparations exhibited the same beefy flavor and odor notes. Ultrafiltration is a technique of preparative isolation, fractionation, and purification having the advantages of simplicity, speed, and economy.

Beef flavor precursors are small molecular weight compounds present in raw meat and are the prime source of the characteristic flavor developed upon processing. These compounds are extractable with cold water along with proteins.

Various inorganic salts, organic and inorganic acids are in use to precipitate proteins for the preparation of a protein-free extract. Trichloroacetic acid is a well known protein precipitant (Neuberg *et al.*, 1944). Some of the most commonly used deproteinizing reagents are acetic acid with heat (Flatow, 1928), tungstic acid (Woodward and Fry, 1932), molybdic acid (Hess, 1929), tungstomolybdic acid (Benedict and Gottschall, 1933), metaphosphoric acid (Fujita and Iwatake, 1935), tetrametaphosphate and other polyphosphates (Pennell, 1960), picric acid (Hamilton and Van Slyke, 1943), sulfosalicylic acid (Hamilton, 1962), and perchloric acid (Neuberg *et al.*, 1944). Each one of these reagents has its specific use, advantages, and disadvantages. The use of trichloroacetic acid has been criticized because of the tendency of glutathione to become autoxidized (Fujita and Iwatake, 1935). Furthermore, trichloroacetic acid removal from solutions is laborious and time consuming. The presence of traces of trichloroacetic acid in the preparation of protein-free nucleotides' extract introduces errors in the nucleotides' content determined by ultraviolet absorption techniques (Hutchison and Munro, 1961). Block *et al.* (1966) stated that the recovery of added amounts of aspartic, threonine, glycine, valine, isoleucine, leucine, tyrosine, phenylalanine, and arginine from picric acid deproteinated plasma pool

Food Laboratory, U. S. Army Natick Laboratories, Natick, Massachusetts 01760.

ranged from 93.1 to 107.5%. Recovery values for serine were 123.9% and for cystine were 69.1%. On deproteination with sulfosalicylic acid of plasma plus added amounts of amino acids, recovery values for proline, glycine, alanine, valine, cystine, methionine, isoleucine, tyrosine, and phenylalanine ranged from 92.7 to 108.8%. On the other hand, the recovery values for aspartic, threonine, serine, glutamic, leucine, lysine, histidine, and arginine ranged from 82.7 to 116.9%. During protein precipitation, trichloroacetic, picric, and sulfosalicylic acids irreversibly denature most proteins.

For chemical analysis and sensory evaluation, deproteinizing reagents must be completely removed from the extract solution. Stein and Moore (1954) removed picric acid by retention on strong anion-exchange resin, Dowex 2X8, in the chloride form and used dilute aqueous HCl solution to elute the extract components from the resin. Zaika (1969) removed picric acid from beef extract by adsorption on Amberlite XAD-2 and eluted the flavor precursors with water. The process of removing the deproteinizing reagents and recovering the flavor precursors intact is laborious and tedious. Zaika (1969) reported that the recovery of guanine and adenine from picric acid solution is not feasible.

Dialysis is the procedure most commonly used to recover beef flavor precursors from aqueous extracts (Mabrouk *et al.*, 1969). The rate of dialysis of a particular solute depends upon many factors: the ratio of membrane area to the solution volume; the nature of membrane, its thickness, porosity, etc.; temperature; the effect of solvent on solute, *e.g.*, association tendency, conformational behavior, and other factors concerned with ideality; the effect of solvent on membrane; possible charge effects and the viscosity of the solvent (Craig and Stewart, 1965; Maxwell and Moffitt, 1965). Goldstein and Craig (1960) reported that during dialysis the majority of peptides were more or less strongly influenced by relatively small concentrations of salt in the solvent. They also found that yeast's RNA, which contains approximately 100 nucleotide units, will readily diffuse through a membrane of suitable porosity when the solvent is a salt solution but will not dialyze at all in salt-free water. Changes in either the hydrogen ion concentration or in the ionic strength of solutions would also markedly influence dialysis rates for many solutes (Craig *et al.*, 1957). Cellulose membranes which are extraordinarily free from fixed charges, even carboxyl groups (Craig and Ansevin, 1963), are generally used in dialysis of aqueous beef extract to obtain beef flavor precursors. Unfortunately, dialysis is a time-consuming, relatively inefficient procedure at low concentrations and produces large volumes of diffusates which require freeze drying for recovering the flavor precursors intact.

During the last 10 years, membrane ultrafiltration has gained increasing prominence as a simple and convenient process for concentrating, purifying, and fractionating solutions of moderate to high molecular weight compounds, and for purifying water and other solvents containing such solutes. The development of this new molecular separation technique for both laboratory and industrial application is attributed to the development of uniquely structured polymeric membranes which display extraordinarily high hydraulic permeabilities coupled with the capacity to retain small molecular weight solutes (Blatt *et al.*, 1970).

The present study was undertaken to investigate the applicability of the ultrafiltration technique to the isolation of beef flavor precursors intact, in high yields, and in a short time. Furthermore, the effect of ultrafiltration environments such as pressure, speed of stirring, and solute concentration on ultrafiltration products was studied.

## EXPERIMENTAL SECTION

**Materials.** *Preparation of Freeze-Dried Aqueous Beef Extracts.* The dry material of aqueous beef extract was prepared according to the method of Mabrouk *et al.* (1969).

*Ultrafiltration Systems.* Two types of ultrafiltration systems were used in this investigation. Batch-type ultrafiltration apparatus (Amicon Corp., Lexington, Mass.) consisted of an Amicon Model 2000 system having a cell designed to accommodate a 150-mm circular membrane with about 162 $cm^2$ effective membrane area and a 20-l. capacity reservoir attached by a dual manifold to a nitrogen gas tank. The reservoir is connected to the cell with stainless steel tubing and needle valve. Thus, the water held in the reservoir provides a volume of dialyzate that progressively removes from the solution compounds of molecular weights below the cut-off point level of the membrane. The cell is equipped with an integral magnetic stirrer to dissipate the retained solutes which otherwise form a cake on the membrane surface and lower the flux. The stirring speed ranges from 0 to 516 rpm. The cell can sustain pressures up to 100 psi and is transparent, so that the progress of ultrafiltration can be followed directly with the naked eye. The cell was always filled with 2000 ml of aqueous beef extract solution and the reservoir was filled with D.I. water. While the needle valve between the reservoir and the cell was closed, nitrogen gas was admitted simultaneously to the cell and the reservoir at 50 psi. Then the stirrer was turned on and the rate of stirring was increased gradually to 426 rpm. Simultaneously, the pressure valve of the cell was closed and the needle valve between the cell and the reservoir was opened. The same starting pressure in both cell and reservoir minimized the volume change in the ultrafiltration cell during operation. As the ultrafiltrate was discharged from the cell, the solution was replaced with D.I. water from the reservoir. Nitrogen pressure (50 psi) served to pump D.I. water through the cell that progressively removed beef flavor precursors from the aqueous extract. The reservoir can also be used when the volume of the solution to be ultrafiltered exceeds the capacity of the cell; thus beef flavor precursors can be prepared directly from the freshly prepared aqueous extract. Two sets of experiments were carried out with this cell.

A total ultrafiltrate volume eight times that of the cell was collected at predetermined intervals and freeze dried; the volume of the ultrafiltrate and the weight of the freeze-dried material were recorded. Several experiments were performed to study the rate of exchange of beef flavor precursor, ultrafiltration flux rates, and flux products. The term flux rate denotes the volume of aqueous beef extract transferred across an ultrafiltrate membrane area perpendicular to the direction of the flow in a given time, g/$ft^2$-D or ml/$cm^2$-min. On the other hand, the term flux product designates the weight of aqueous beef extract solids transferred across a given membrane area perpendicular to the direction of the flow in a given time, kg/$ft^2$-D, g/$cm^2$-hr, g/$cm^2$-sec, or mg/$cm^2$-min. For these studies, the ultrafiltrate was monitored for its ultraviolet absorption at 280 m$\mu$ using GME ultraviolet absorption meter (Gilson Medical Electronics, Middleton, Wash.) equipped with recti/riter recorder (Texas Instruments, Inc., Houston, Tex.). Twenty-two milliliter aliquots of the ultrafiltrate were collected in a GME fraction collector. Thus, ultraviolet absorption of the ultrafiltrate was monitored as a function of ultrafiltrate volume which emerged from the cell. Ultrafiltration was considered complete when ultraviolet absorption was zero. To ensure that ultrafiltration was complete, two 1-l. fractions were collected and freeze-dried and the residues were weighed. The weight of the residue of each fraction was less than 15 mg.

Each ten fractions obtained with the fraction collector, *i.e.*, 220 ml, were combined, shell frozen, and freeze dried, and the weight of the freeze-dried material was recorded. The time of collecting each ten fractions was calculated from the chart chromatogram.

Recirculating thin-channel system (Amicon Corp., Lexington, Mass.) was composed of a cell and peristaltic pump. This system uses advanced fluid management concepts with high velocity laminar flow in narrow channels of 0.25-mm depth and an effective membrane area of 138 $cm^2$. The cell was pressurized with nitrogen gas at 50 psi. The peristaltic pump with an internal volume of 220 ml and a hold-up volume of 5 ml was used to drive the solution through the spiral channel in an insert at the base of the cell. When this system was run in the cold room at 5°, the peristaltic pump did not function properly. Therefore, the recirculating thin-channel system experiments were carried out in the laboratory by keeping the glass cylinder containing 40 g of freeze-dried aqueous beef extract dissolved in 1000 ml of D.I. water immersed in a wet ice bath. The ultrafiltrate was collected in a GME fraction collector as stated above. Each 22-ml fraction was shell frozen immediately after collection in the fraction collector.

*Ultrafiltration Membranes.* Diaflo membranes (Amicon Corp., Lexington, Mass.) types UM-10 and UM-20E which restrict the passage of compounds in excess of 10,000 and 20,000 molecular weight, respectively, were used in this study.

*Factors Affecting Ultrafiltration.* The effect of varying the rate of stirring speed, pressure, and different concentrations of beef extract on ultrafiltration flux was investigated. This study was done with the same cell and with the same membrane. After each run, the membrane was washed well with D.I. water, 50% aqueous ethanol, and D.I. water was ultrafiltered through it for 1 hr. The flux rate of D.I. water obtained with the reused membrane was ±3% of the original value.

*Gel Permeation Chromatography (gpc).* Sephadex three columns set, each 2.2-cm i.d. by 100 cm in length, were arranged in series. The columns were connected with spaghetti Teflon tubing 2.3-mm i.d. The first column was packed with G-10, the second contained G-15, and the third was charged with G-25. About 1.2 g of beef flavor precursors concentrate dissolved in 20 ml of 0.05 *M* ammonium formate–formic acid buffer solution, pH 5.00, was injected into the first column (G-10). Elution of Sephadex beds was performed at a flow rate of 54 ml of formate buffer/hr. The ultraviolet absorption of the effluent was recorded using a GME ultraviolet absorption meter equipped with Texas Instrument recti/riter recorder. All gpc experiments were performed in the cold room at 5°.

## RESULTS AND DISCUSSION

**Parameters Affecting Ultrafiltration.** The effect of speed of stirring, pressure applied, and solute concentrations of aqueous beef extract solutions on ultrafiltration products in a 2000-ml capacity batch cell is summarized in Table I.

The flux products of beef extract with Diaflo membranes (UM-20E) with high fluxes of pure water increased with increasing the concentration of the solution, the speed of stirring, and the pressure applied. While the flux rates ($ml/cm^2$-min) decreased with increasing concentration of beef extract, increasing either the rate of stirring or the pressure in the system caused an increase in the flux rates. This is in agreement with the findings of Baker and Strathmann (1970) and Porter and Michaels (1971).

**Conventional Dialysis *vs.* Dialysis by Ultrafiltration "Diafiltration."** Diafiltration is the continuous process of solute exchange by the addition of fresh solvent; it is also called "Rapid Dialysis."

Using Oxford multiple dialyzer which accommodated 16 cellulose sacs (Viscose process, size 27, 33.325-mm flat, width, 0.0254-mm wall thickness and average bore radius of 24 A) each containing 250 ml of D.I. water rotating at 10 rpm in 14 l. of aqueous beef extract for 10 days with intermittent change of sacs' contents resulted in collecting

**Table I. Effect of Pressure Applied, Rate of Stirring, and Solute Concentration on Ultrafiltration of Aqueous Beef Extract, UM-20E Membrane**

| Beef extract, g/l. | Rate of stirring, rpm | Pressure, psi | Flux product, $mg/cm^2$-min | Flux rate, $ml/cm^2$-min |
|---|---|---|---|---|
| 20.25 | 516 | 50 | $6.23 \times 10^{-2}$ | $2.70 \times 10^{-2}$ |
| 30.1 | 516 | 50 | $17.69 \times 10^{-2}$ | $1.62 \times 10^{-2}$ |
| 40.0 | 516 | 50 | $24.00 \times 10^{-2}$ | $1.22 \times 10^{-2}$ |
| 40.0 | 264 | 50 | $12.30 \times 10^{-2}$ | $0.68 \times 10^{-2}$ |
| 40.0 | 516 | 30 | $18.80 \times 10^{-2}$ | $0.83 \times 10^{-2}$ |

**Table II. Percent Beef Diffusate Recovered with Conventional Dialysis and Diafiltration at 5°**

| | % beef diffusate recovered[a] | | |
|---|---|---|---|
| | | Diafiltration | |
| Time, hr | Conventional dialysis | UM-10 | UM-20E |
| 20.0 | 17.29 | 70.0 | 62.33 |
| 25.0 | 26.41 | 76.04 | 67.13 |
| 45.0 | 37.90 | 88.47 | 80.39 |
| 64.0 | 46.53 | 93.00 | 87.88 |
| 85.5 | 56.02 | 96.00 | 92.72 |
| 107.5 | 66.63 | 98.88 | 98.95 |
| 134.0 | 71.21 | | |
| 158.0 | 77.80 | | |
| 163.2 | 82.55 | | |
| 185.5 | 87.97 | | |

[a] Beef diffusate obtained with conventional dialysis; ultrafiltrations through UM-10 membrane and through UM-20E membrane were 49.08, 49.08, and 53.28% of the aqueous extract, respectively.

40 l. of beef diffusate solution which contained only 88% of the total diffusible material. A comparison between the yields obtained with conventional dialysis and diafiltration techniques at predetermined intervals is presented in Table II.

While dialysis is time consuming, the chances for error are inherent because of the manipulations involved; diafiltration is less time consuming, requires fewer manipulations, and gives the highest yield. The speed of diafiltration in comparison with dialysis could be attributed to the fact that the Diaflo membranes used have a much higher water permeability relative to that of cellulose (Blatt *et al.*, 1967). While it took 10 days to recover 88% of beef diffusate with conventional dialysis, the same effect was achieved in about 2 to 2.5 days using the high flow cell Model 2000. Upon using the continuous flow recirculating thin-channel system, the same effect was achieved in 6 hr, *i.e.*, a fraction of the time (2.5%) needed for conventional dialysis. 17.27616 g of diffusate was recovered from 40 g of freeze-dried aqueous beef extract dissolved in 1000 ml of D.I. water with a thin-channel system equipped with a UM-20E membrane. Upon the addition of D.I. water to the glass cylinder to recover the diffusible material in the aqueous beef extract solution held in the internal volume of the peristaltic pump, 4.18539 g of beef diffusate was collected. Thus, the total beef diffusate amounted to 21.46155 g, *i.e.*, 53.65% of the aqueous extract. This demonstrates that the agitation in batch cells is inferior to the high velocity laminar flow in the narrow channels concept used in thin-channel system.

Figure 1 indicates that in the case of beef diffusate, 81 to 90% recovery was achieved with UM-10 and UM-20E membranes, respectively, when the water passage through the system corresponds to three times the cell volume. Ninety-six percent removal of beef diffusate occurred with a four-volume turnover, and 99% recovery was achieved with five times the original volume of beef extract in a batch cell equipped with UM-20E. In the case of the

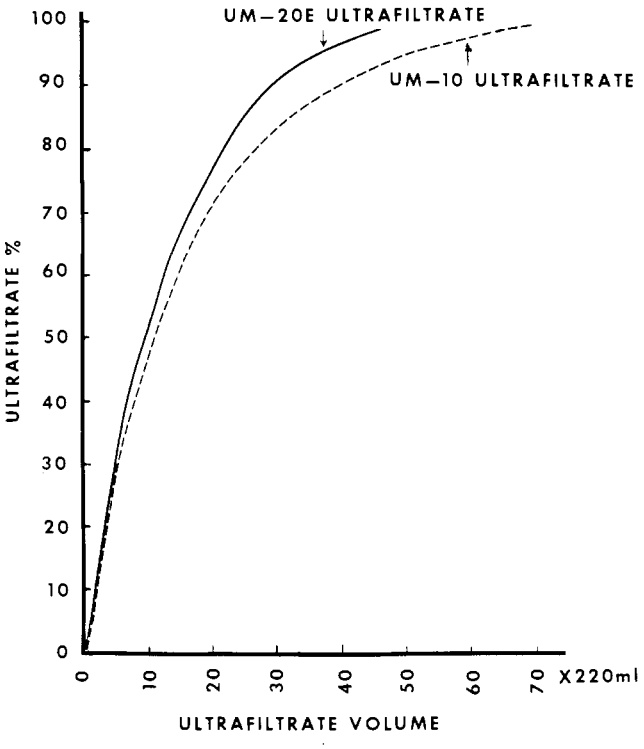


**Figure 1.** Percent beef ultrafiltrate recovered with respect to ultrafiltrate volume.

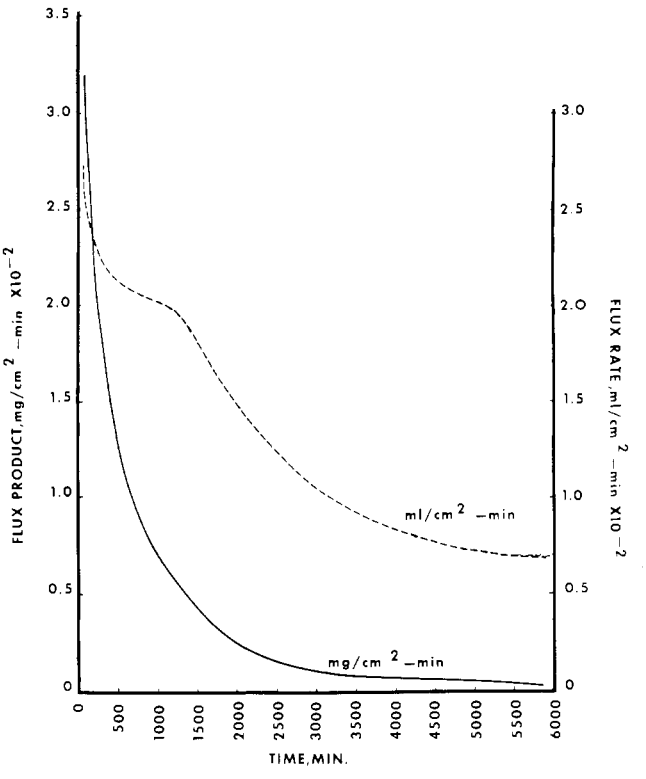


**Figure 2.** Ultrafiltration flux of beef flavor precursors with UM-10 membrane in stirred cell.

UM-10 membrane, about 7.4 times the original volume was required to recover 99% of the diffusible material in the ultrafiltrate. With high flow cell model 2000, the amount of beef diffusate recovered increased with increasing the total volume of diafiltrate.

In the presence of the UM-20E membrane, the diffusate yield in grams is higher than that of the UM-10 membrane due to the difference in molecular weight cut-off. This is manifested in the gpc large peak of the void volume of the diffusate obtained with the UM-20E membrane in comparison with that of the UM-10 membrane.

**Selective Ultrafiltration of Aqueous Beef Extract.** The ultrafiltration fluxes of beef flavor precursors with UM-10 and UM-20E membranes are demonstrated in Figures 2 and 3, respectively.

The flux rates of aqueous beef extract solutions were much lower than the experimental values of D.I. water flux, 17.00 × $10^{-2}$ and 24.07 × $10^{-2}$ ml/cm²-min for UM-10 and UM-20E, respectively. These are in agreement with the findings of van Oss (1968), who reported that the rate of ultrafiltration of 5% protein solution was reduced to anywhere between 9 to 29% of the flux of pure water. The experimental results given in Figures 2 and 3 indicate that flux decreased gradually with time. This reduction in flux is attributed to the poor agitation in batch cells, thus resulting in accumulation of retained solutes at the membrane surface. This phenomenon is known as "concentration polarization" (Michaels, 1968).

After 4000 min, the agitation of the solution in the cell equipped with UM-20E membrane (Figure 3) caused dissipation of the retained solutes at the membrane surface, thus causing an increase in the flux rate from 0.5 × $10^{-2}$ to 1.44 × $10^{-2}$ ml/cm²-min, consequently increasing the flux product from 1.0 × $10^{-2}$ to 1.5 × $10^{-2}$ mg/cm²-min. In the recirculating thin-channel system, the reduction of concentration polarization had been achieved as circulation of fluid through channels of small depth produced sufficient shear to reduce the boundary layer and increase solvent flow. High ultrafiltration rates 3.2 × $10^{-2}$–2.4 × $10^{-2}$ ml/cm²-min were achieved with the circulating thin-channel system (Figure 4).

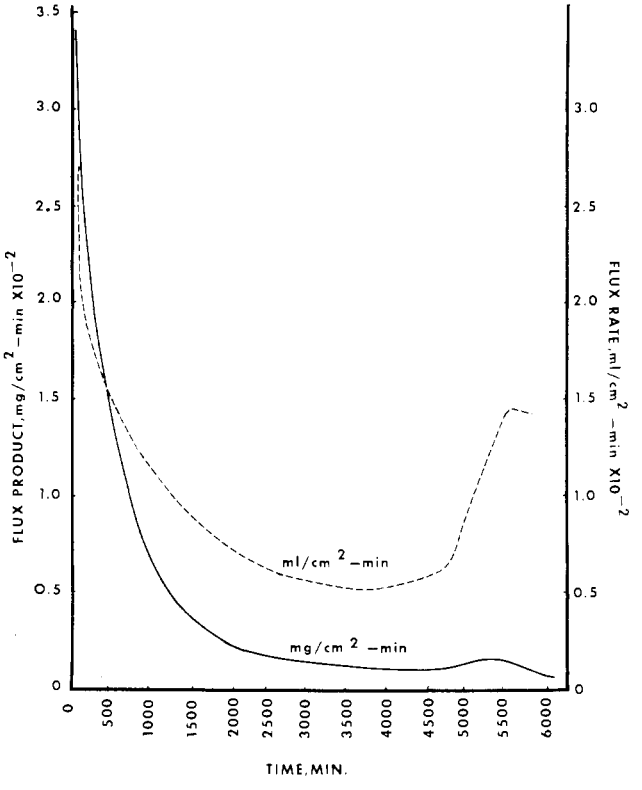


**Figure 3.** Ultrafiltration flux of beef flavor precursors with UM-20E membrane in stirred cell.

Figure 4 indicates the flux products ranged between 54.33 and 35.90 × $10^{-2}$ mg/cm²-min during the first 880 ml of ultrafiltrate. Upon the addition of 220 ml of water to the cylinder to continue ultrafiltration of the pump internal contents, the flux products ranged from 35.90 to 22.74 × $10^{-2}$ mg/cm²-min. Upon further addition of 220 ml of water, the flux products decreased and varied between 22.45 and 15.26 × $10^{-2}$ mg/cm²-min. The observed de-

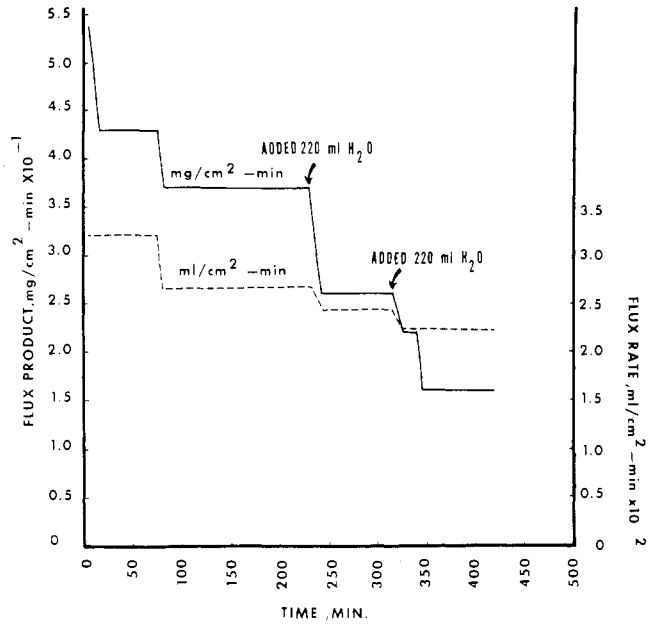


**Figure 4.** Ultrafiltration flux of beef flavor precursors with UM-20E membrane in recirculating thin-channel system.

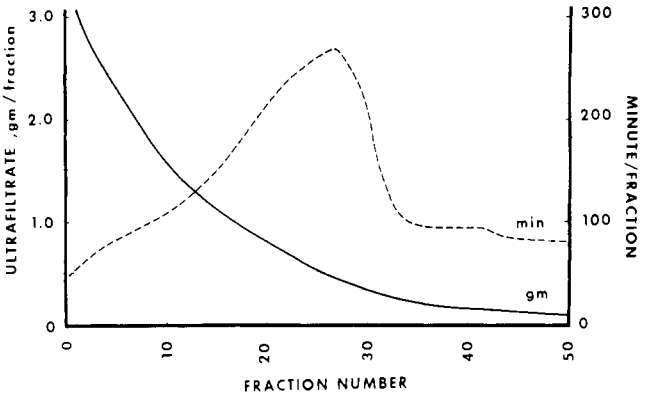


**Figure 5.** Diafiltration of beef extract with UM-20E membrane in stirred cell, 4% starting solution.

crease in flux rates (ml/cm²-min) upon the addition of each 220 ml of water aliquot may be attributed to the presence of a very thin film of precipitation on the ultrafiltration membrane surface, which was noticed upon disassembling the cell at the end of each experiment. The observed variations in flux products were due to change in concentration upon the addition of water to recover the beef diffusate in the pump internal volume. This substantiates our findings (Table I) that flux product is a function of solute concentration in solution. Recirculating thin-channel ultrafiltration systems give a high yield in shorter time than the batch system; thus, these systems are more economical. Comparing the data in Figure 4 with those in Figure 3, it is clear that when the same type of membrane was used in the batch cell it had lower flux rates than when used in a thin-channel system. The thin-channel system data (Figure 4) are, on the average, three- to tenfold those of the batch cell (Figure 3). For the duration of the ultrafiltration period of 4% solids aqueous beef extract solution, the thin-channel system equipped with UM-20E membrane retained a narrow range flux rate 31.88–22.77 × $10^{-2}$ ml/cm²-min. This small variation is due mainly to the fluctuation of solute concentration as ultrafiltration progressed. Figures 5 and 6 show the performance of a stirred batch cell equipped with UM-20E and UM-10 membranes, respectively, for ultrafiltration of beef extract solution (4% solids).

In the case of UM-20E membrane, 3.07 g of diffusate was collected in the first 220-ml ultrafiltrate (first fraction). As ultrafiltration proceeded, the concentration of diffusate per fraction decreased gradually and leveled off when 99% of the diffusate was collected (fraction ≈45). While the first fraction was collected in about 50 min, the time needed to collect the same volume of ultrafiltrate increased gradually and leveled off at fraction ≈43. The same pattern was observed with the UM-10 membrane (Figure 6).

**Gel Permeation Chromatography (gpc) of Beef Diffusate.** The gpc chromatograms of beef diffusates obtained with conventional dialysis and diafiltration through UM-10 and UM-20E membrane are identical beyond the void volume fraction (Figure 7).

From Figure 7 it is evident that there is great similarity between the beef flavor precursors prepared by ultrafiltration and that recovered by conventional dialysis. Flavor and odor evaluations indicated that the three preparations exhibited the same flavor and odor. These results are in agreement with the findings of Blatt *et al.* (1967), who reported that diffusivity of a given protein does not seem to be altered by using a protein mixture. Gel permeation

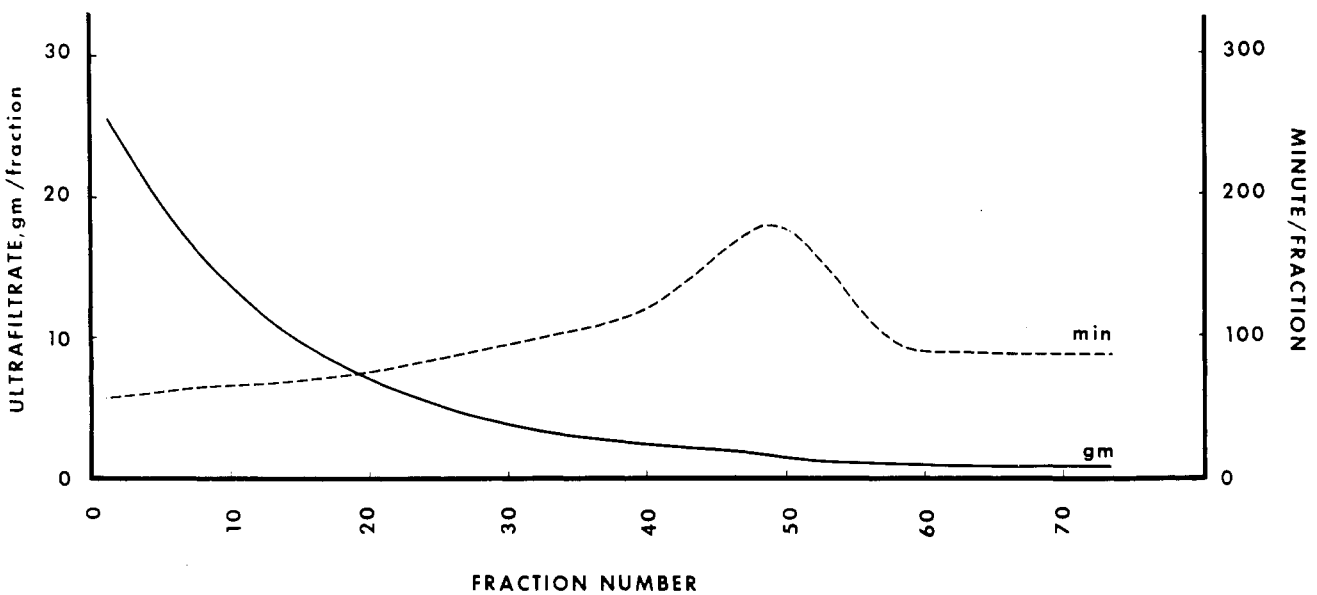


**Figure 6.** Diafiltration of beef extract with UM-10 membrane in stirred cell, 4% starting solution.

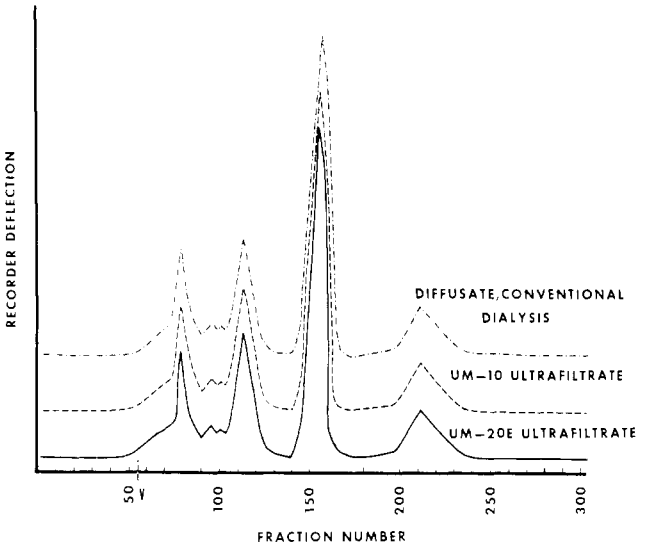


**Figure 7.** Gel permeation chromatography of beef diffusates obtained with conventional dialysis and diafiltration through UM-10 and UM-20E membranes.

chromatograms of beef diffusates obtained with either batch cell or recirculating thin-channel system equipped with a UM-20E membrane were identical.

In conclusion, ultrafiltration systems are well suited for isolating beef flavor precursors from beef extract. With ultrafiltration systems available for laboratory use, isolation of beef flavor precursors was achieved in 2.5 to 10% of the time necessary for conventional dialysis. Following the manufacturer's recommendations on membranes care, we were able to use the same membrane continuously for a month without noticeable reduction in its flux rates. Thus, the operating cost of ultrafiltration systems is far lower than that of conventional dialysis. With the type of available membranes, separation of individual compounds is not feasible, but as membrane technology improves, a group separation of narrow range of molecular size will be achieved.

The excessive volume of ultrafiltrate obtained with diafiltration can be reduced by concentrating the ultrafiltrate by using another cell containing the UM-05 membrane (500 molecular weight cut-off). Thus, two cells in tandem assembly equipped with UM-10 and UM-05 membranes should be used. Water washes out low molecular weight compounds through the two membranes. At the end of the run, three fractions are recovered; one contains compounds of molecular weight higher than 10,000, while the second has compounds of molecular weights ranging between 500 and 10,000, and the third includes compounds of molecular weights below 500. Thus, the recovered beef flavor precursors (in the cell equipped with UM-05) are free from low molecular weight compounds, *e.g.*, amino acids, amines, sugars, organic acids, etc. This fraction could be concentrated in the cell to 50 ml, thus minimizing the time of freeze drying. Upon heating dry, each fraction contributed specific flavor notes.

In reality, ultrafiltration provides the flavor chemist with an inexpensive rapid means for the nondenaturing separation of beef flavor precursors and any labile compound.

## LITERATURE CITED

Baker, R. W., Strathmann, H., *J. Appl. Polym. Sci.* **14**, 1197 (1970).
Benedict, S. R., Gottschall, G., *J. Biol. Chem.* **99**, 729 (1933).
Blatt, W. F., Dravid, A., Michaels, A. S., Nelson, L., "Membrane Science and Technology," Plenum Press, New York, N. Y., 1970, pp 47-97.
Blatt, W. F., Hudson, B. G., Robinson, S. M., Zipilivan, E. M., *Nature (London)* **216**, 511 (1967).
Block, W. D., Markovs, M. E., Steele, B. F., *Proc. Soc. Exp. Biol. Med.* **122**, 1089 (1966).
Craig, L. C., Ansevin, A., *Biochemistry* **2**, 268 (1963).
Craig, L. C., Stewart, K., *Biochemistry* **4**, 2712 (1965).
Craig, L. C., King, T. P., Stracher, A., *J. Amer. Chem. Soc.* **79**, 3729 (1957).
Flatow, L., *Biochem. Z.* **194**, 132 (1928).
Fujita, A., Iwatake, D., *Biochem. Z.* **277**, 284 (1935).
Goldstein, J., Craig, L. C., *J. Amer. Chem. Soc.* **82**, 1833 (1960).
Hamilton, P. B., *Ann. N. Y. Acad. Sci.* **102**, 55 (1962).
Hamilton, P. B., Van Slyke, D. D., *J. Biol. Chem.* **150**, 231 (1943).
Hess, W. C., *J. Wash. Acad. Sci.* **19**, 419 (1929).
Hutchison, W. C., Munro, H. N., *Analyst (London)* **86**, 768 (1961).
Mabrouk, A. F., Jarboe, J. K., O'Connor, E. M., *J. Agr. Food Chem* **17**, 5 (1969).
Maxwell, M. M., Moffitt, R. A., *Anal. Biochem.* **11**, 566 (1965).
Michaels, A. S., in "Progress in Separation and Purification," Perry, E. S., Ed., Vol. 1, Interscience Publishers, New York, N. Y., 1968, pp 297-334.
Neuberg, C., Strauss, E., Lipkin, L. E., *Arch. Biochem.* **4**, 101 (1944).
Pennell, R. B., "The Plasma Proteins," Putnam, F. W., Ed., Vol. 1, Academic Press, New York, N. Y., 1960, p 9.
Porter, M. C., Michaels, A. S., *Chem. Technol.* **1**, 56 (1971).
Stein, W. H., Moore, S., *J. Biol. Chem.* **211**, 915 (1954).
van Oss, C. J., "Progress in Separation and Purification," Perry, E. S., Ed., Vol. 1, Interscience Publishers, New York, N. Y., 1968, p 229.
Woodward, G. E., Fry, E. G., *J. Biol. Chem.* **97**, 465 (1932).
Zaika, L. L., *J. Agr. Food Chem.* **17**, 893 (1969).

Received for review May 29, 1973. Accepted August 29, 1973. Research was undertaken at the U. S. Army Natick Laboratories and has been assigned No. TP-1373 in the series of papers approved for publication. The findings in this report are not to be construed as an official Department of the Army position. Mention of trade product, equipment, or company does not imply endorsement by the U. S. Army Natick Laboratories over similar products or companies not mentioned.