FRACTIONATION AND RECOVERY OF COTTONSEED WHEY CONSTITUENTS BY ULTRAFILTRATION AND REVERSE OSMOSIS

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ABSTRACT

Isolation of protein from cottonseed and other oilseed flours results in a whey-type liquid by-product. Cottonseed wheys contain from 21 to 31% of the nitrogen in the flour extracted. To recover valuable constituents from these wheys and solve the disposal problem they present, the wheys were processed using ultratiltration (UF), and reverse osmosis (RO) membranes. In a typical pilot plant scale run, membrane processing lowered the chemical oxygen demand (COD) from 12×10^3 p.p.m. to 65 p.p.m. Total solids content of whey was reduced by more than 99% in the effluent from the RO membrane. COD was found to correlate closely with total solids minus ash (correlation coefficient = 0.991) and protein plus carbohydrates (correlation coefficient = 0.992). Concentrated constituents retained by the UF membrane when spray dried yielded high-protein products which had commercial potential either as whipping agents or for use in protein fortification of breads and beverages. The composition and yields of these products were determined. Concentrates containing salts and carbohydrates retained by the RO membrane were also spray dried and product composition and yields determined.

The liquid wheylike by-products from protein isolation from cottonseed flours are expected to command a major share of the processor's attention for two reasons: 1) these wheys retain a significant portion of the protein and nutrients extracted from the flour, and 2) they constitute a serious pollution problem when discarded into domestic water bodies without proper treatment. For each pound of protein isolated from cottonseed flour 40 to 95 lb. of whey are produced depending on the isolation process employed. The wheys contain from 21 to 31% of the original flour nitrogen.

Cheese wheys, a perennial economic liability and pollutant, are now being processed at a profit with semi-permeable ultrafiltration (UF) and reverse osmosis (RO) membranes. Valuable food ingredients are being recovered and pollution potential is being nullified simultaneously (1,2). Also, soy whey, which is similar to cottonseed whey, has been successfully processed with membranes (3).

In research in progress at the Texas A&M University Food Protein Research and Development Center (FPRDC) membrane processing techniques have been applied to cottonseed wheys derived from three different isolation processes. A concept of recycling the effluent from the second, or RO, step in the processing for reuse in flour extraction has been demonstrated to be feasible (4). Spray-dried protein products from ultrafiltration of cottonseed wheys have also been tested in a number of food applications and demonstrated to have potential for commercial acceptability (5).

In the work reported herein, both UF and RO membrane operating characteristics with cottonseed wheys were studied, but principally those of the UF membrane. The yields and compositions of constituent products recovered were determined.

MATERIALS AND METHODS

Preparation of Wheys

Three protein isolation procedures described previously (6) were used to prepare cottonseed wheys for study. Multiple runs were made with each procedure in the FPRDC pilot plant. Forty pounds of Rogers GL-7 glandless cottonseed flour was extracted in each run.

Five different wheys result from the three isolation procedures used. One procedure, designated process B, yields a single whey. Products from that whey are identified as UF B and RO B according to the membrane used to recover them.

A second isolation procedure, process C, produces two wheys identified as C-SP and C-NSP. These wheys result from two separate extraction steps in process C in which storage protein and nonstorage protein are extracted separately and precipitated separately.

The third extraction procedure, designated process F, also produces two different wheys. One whey which results from precipitation of the extracted protein at pH 3 is identified as F-major. The other whey which results from resuspending the curd precipitated at pH 3 in tap water at pH 7 followed by centrifuging is identified as F-minor.

Composition of the tap water used in whey preparation is given in Table I since variability of ash in water from different geographic locations influences whey composition.

Processing of Wheys

Each whey, irrespective of isolation procedure, was pasteurized by heating to 145° F. for 30 min. and then fed unfiltered into UF membranes at 115° to 120° F. The FPRDC pilot plant is equipped with a ROpak Single-Core Reverse Osmosis machine manufactured by Ray Pak, Inc., Westlake Village, Calif. This UF/RO machine contains 24 sq. ft. of tubular type UF membranes (5,000 to 10,000 MW cut off) and 24 sq. ft. of RO membranes (90% NaCl rejection). Its cellulose-based membranes are supported on the exterior of 5/8 in. diameter ceramic cores. The rod-like membrane cores housed in 6-ft. long stainless-steel tubes allow passage

TABLE I. TYPICAL COMPOSITION OF TAP WATER USED IN PROTEIN ISOLATION PROCESSES AT FPRDC[1]

Components	Concentration p.p.m.
Calcium	1.5
Magnesium	0.5
Sodium	130.0
Chloride	47.0
Bicarbonate	299.0
Silicate	15.0

[1]Conductivity = 727 μmhos.; pH = 8.4.

of salts and carbohydrates and low-molecular-weight nitrogenous substances into the UF filtrate (permeate) and retain and concentrate protein constituents. UF permeate is then fed into a bank of RO membranes. These membranes concentrate salts and carbohydrates passing water and about 10% of the salts.

In the ultrafiltration step, whey was fractionated and concentrated by batches. The original volume (usually 60 to 75 gals.) was reduced by four-fifths. It was then diluted with a volume of tap water equal to the remaining one-fifth and concentrated again to one-fifth of the original volume to achieve the equivalent of a 10-to-1 volume reduction for the original whey. UF concentrate was spray-dried with an Anhydro Spray Dryer, Type III-A, No. 2. Inlet air temperature of 300° to 310°F. and outlet air temperature of 185° to 195°F. were used.

A sufficient quantity of RO concentrate was freeze-dried for making yield calculations and analytical determinations on dry RO product.

The effects of solids concentration, protein concentration, and viscosity in feed to the UF membranes on UF permeate flux rate were determined by taking samples of UF feed and the corresponding flux rates at constant temperature and pressure over a period of several hours.

Differential experiments in which UF permeate was returned to the feed tank to keep feed concentration and composition constant were made to study the effect of temperature on permeate flux rate.

Percentages of whey components retained by membranes were calculated as follows:

$$\% \text{ Retention of Component} = \frac{(\text{Conc.}_{\text{Feed}} + \text{Conc.}_{\text{Conc.}})/2 - \text{Conc.}_{\text{Permeate}}}{(\text{Conc.}_{\text{Feed}} + \text{Conc.}_{\text{Conc.}})/2} \times 100$$

Analytical Measurements

Conductivity of wheys and whey fractions was measured with a Model 31 Conductivity Bridge manufactured by the Yellow Springs Instrument Co., Inc.,

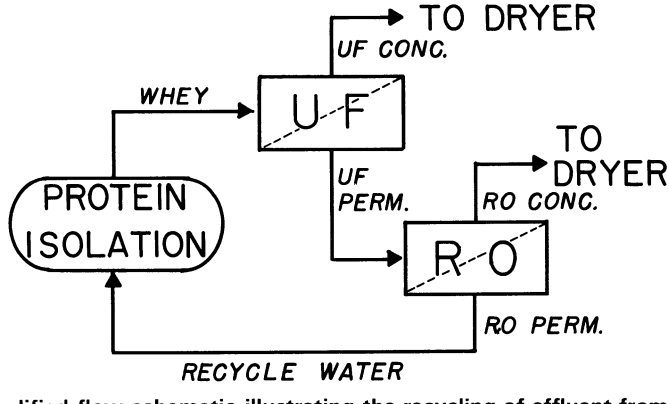

Fig. 1. Simplified flow schematic illustrating the recycling of effluent from membrane processing of cottonseed wheys.

Yellow Springs, Ohio. Total nitrogen was determined by either the macro- or micro-Kjeldahl method. Nonprotein nitrogen (NPN) was determined as nitrogen soluble in 10% TCA solution.

Carbohydrates in terms of glucose were measured colorimetrically by a phenol-sulfuric acid method (7). Chemical oxygen demand (COD) was determined using a rapid method by Jeris (8).

Amino acid analyses of products studied (with the exception of tryptophan and cystine) were quantitatively determined by the procedure developed by Spackman et al. (9). Tryptophan was determined by the method of Kohler and Palter (10). Cystine was measured using a modification of a procedure by Schram et al. (11).

Samples were hydrolyzed for determination of all amino acids except cystine and tryptophan in constant-boiling HCl for 24 hr. under a nitrogen flush. Procedures for preparing protein hydrolysate for cystine and tryptophan are specified in the methods cited.

Total and inorganic phosphorus were determined by the method according to Sumner (12). Moisture, oil, crude fiber, ash, and free and total gossypol were determined according to standard AOCS methods (13).

Viscosities of whey solutions were measured with a standard Ubbelohde-type ASTM D445 viscometer at a constant temperature of 120° ± 0.02°F.

RESULTS AND DISCUSSION

The processing of cottonseed wheys by membranes as reported herein has some desirable features which make it attractive, such as the ability to achieve fractionation as well as concentration of constituents without a phase change through the use of heat. However, in a commercial operation an alternate or additional means of concentration beyond the UF membrane may prove desirable. Also, it is anticipated that the RO product would be dried by a less expensive method than spray-drying.

The concept of recycling the effluent from the second or RO stage of membrane processing is illustrated in the simplified sketch shown in Fig. 1. Recycleable water was in excess of one-half of the total water used in the extraction and dilution steps of the isolation procedures. By further concentrating the UF and RO feed solutions before drying an even larger percentage of the total water required in the isolation phase could be provided by recycle water. The recycle procedure could eliminate water pollution and drastically reduce water requirements.

TABLE II. FRACTIONATION AND REMOVAL OF THE CONSTITUENTS IN PROCESS B WHEY BY ULTRAFILTRATION AND REVERSE OSMOSIS MEMBRANES DURING PILOT PLANT RUN

Process Samples	Total Solids %	COD p.p.m.	Nitrogen %	Carbohydrates %	Ash %
Original Whey (Feed to UF)	1.64	12×10^3	0.0936	0.589	0.410
Permeate from UF (Feed to RO)	0.99	6.7×10^3	0.0241	0.407	0.356
Permeate from RO	0.01	65	0.0003	0.0026	0.0055

Membrane Performance

Table II shows components of process B whey before processing and the degree of fractionation and removal of these constituents achieved as the whey is forced sequentially through the UF and RO membranes. More than 99% of the solids in the original whey were removed by the two membranes. The COD was reduced from 12×10^3 p.p.m. to 65 p.p.m.

Table III reveals membrane performance relative to each type of whey constituent. Percentage retention of solids, ash, nitrogen, NPN, carbohydrates and COD are given for each of the five wheys. The UF membrane passed from 60 to 15% of the solids depending upon the composition of a particular whey. The largest percent retention of solids occurred as expected with the C-SP whey which is derived from precipitation of higher-molecular-weight storage protein curd. Eighty-seven percent of the ash in process B whey passed through the UF membrane and only 56% of ash in process F-Major whey. Extraction of protein with a $CaCl_2$ solution in process F results in wheys with abnormally high ash contents and a portion of the ash (calcium ion) is bound to protein molecules which will not pass membrane pores. From 75 to 95% of whey nitrogen was retained in the UF concentrate and 30 to 64% of the carbohydrates.

RO membranes removed essentially all the remaining solids from process B and C wheys and only a slightly lower percentage from process F wheys. RO permeates contained only 0.01% solids.

Figures 2, 3, and 4 contain data taken to determine the effect of certain operating parameters and whey characteristics on UF membrane performance. The sensitivity of UF permeate flux rate to temperature of the feed to UF membranes (process B whey) is shown in Fig. 2. These data were taken with pressure, and feed composition held invariant. Flux rate was measured at 120° F. initially and successively at 10° F. lower intervals to 70° F. after equilibrating feed temperature at each point. After measurement at 70° F., the feed was heated to 120° F. and the series of measurements repeated. Approximately 2 hr. elapsed between the two measurements taken at each temperature but the flux rate

TABLE III. RETENTION OF CONSTITUENTS IN COTTONSEED WHEYS BY ULTRAFILTRATION MEMBRANES AND IN ULTRAFILTRATION PERMEATE BY REVERSE OSMOSIS MEMBRANES

Membrane Type	Whey Type	Solids %	Ash %	Nitrogen %	NPN %	Carbohydrates %	COD %
	B	40	13	75	70	30	54
Ultrafiltration	C-NSP	60	19	87	80	43	35
	C-SP	85	36	95	80	64	73
	F-Major	74	44	90	70	59	87
	F-Minor	73	43	86	90	46	87
	B	99	98	99	100	99	90
Reverse osmosis	C-NSP	99	99	99	...	100	86
	C-SP	99	99	100	...	100	98
	F-Major & Minor Combined	96	96	99	...	99	99

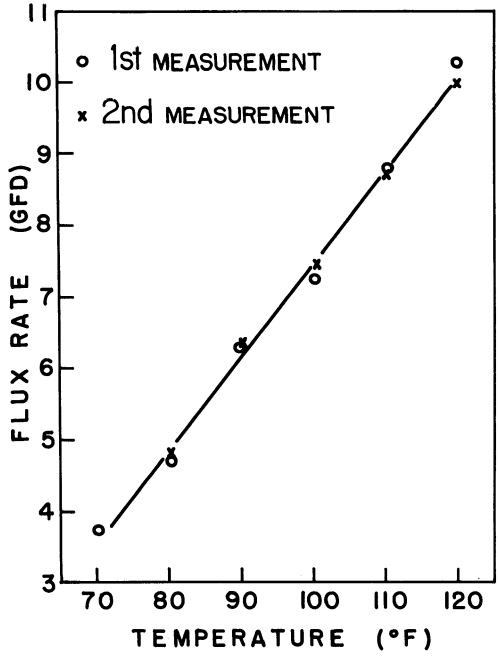

Fig. 2. Effect of whey temperature on the flux rate from UF membranes using process B whey.

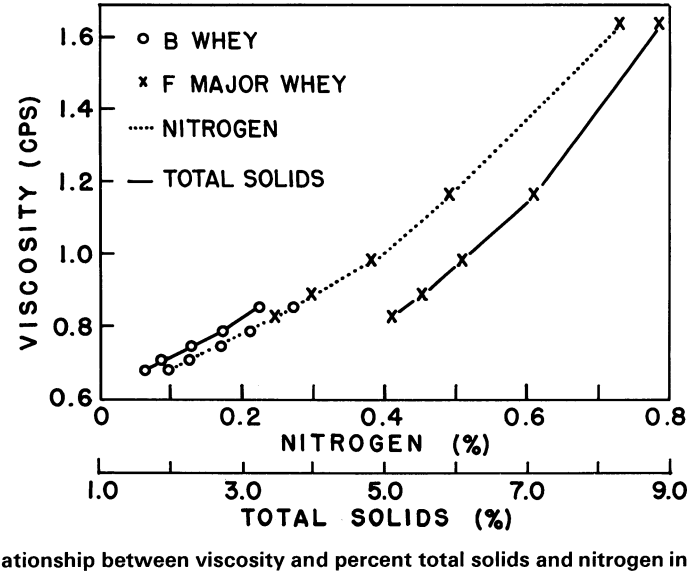

Fig. 3. Relationship between viscosity and percent total solids and nitrogen in process B and process F major wheys.

essentially returned to its former level in each instance. Flux rate responded to feed temperature changes more significantly than to pressure and feed flow rate. A maximum temperature of 120°F. was considered to be safe for use with the cellulose-based membranes.

Figure 3 contains viscosity data on process B and process F-Major wheys. Five samples were taken at 1-hr. intervals from the UF membrane feed tank during processing. Viscosity was found to correlate more closely with nitrogen in the

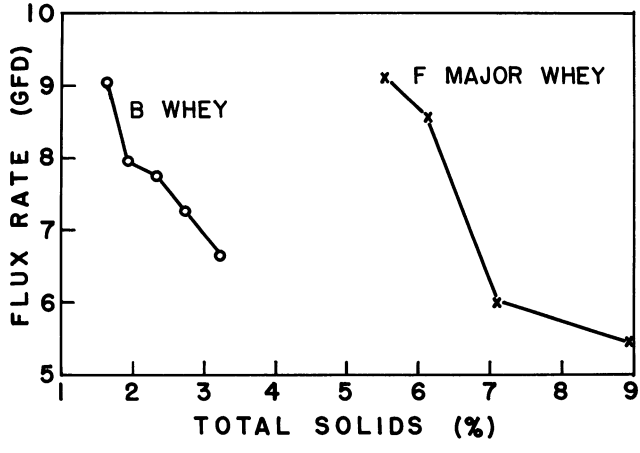

Fig. 4. Relationship between UF permeate flux rate and percent total solids using process B and process F major wheys.

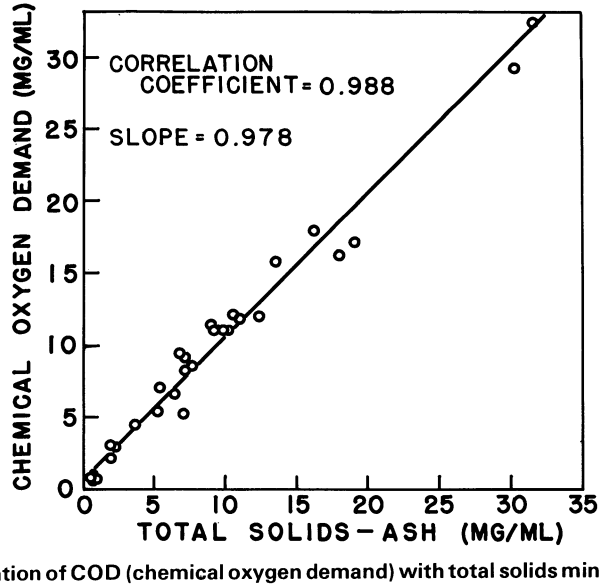

Fig. 5. Correlation of COD (chemical oxygen demand) with total solids minus ash with five wheys from three isolation procedures included in the correlation.

feed than with percent total solids, or total solids minus ash in the feed. As indicated in Fig. 3, viscosity was not the same for the same percent solids in different wheys but tended to be the same for a common percent N in samples regardless of whey source. Viscosity was found to decrease approximately 0.02 cp. per degree centigrade rise in temperature.

Figure 4 depicts UF permeate flux rate versus percent solids for process B and process F-Major wheys. It is evident that flux rate does not correspond to percent solids in feed whey independent of whey source. These two wheys were processed at comparable operating temperatures but were slightly different in pH. As previously stated, process F-Major whey is much higher in ash content than process B whey. They contain 1.83 and 0.41% ash, respectively.

COD has been found to be correlated highly with total solids minus ash regardless of whey source. Figure 5, constructed from data on all five wheys from the three isolation procedures, presents this correlation. Using the regression line shown, the COD of a sample can be predicted from relatively simple percent total solids and percent ash determinations.

Product Yields and Composition

Yields of products from each whey are given in Table IV along with related information. Product yields from each ton of flour extracted and from each 1,000 lb. of whey processed are shown. The first column indicates that 33 lb. of whey were produced for each pound of flour extracted using process C. The different amounts of whey produced per pound of flour result from the differing natures of the isolation processes, chiefly the water-to-solids ratios used in their flour extraction steps. Different degrees of constituent fractionation and different percent solids in wheys result in the variance in product yields. The last column shows the quantity of UF product recovered relative to the total protein isolate recovered for each process.

Analytical data on the dry whey products from each type membrane are presented in Table V. With the exception of the C-NSP product, each UF product was sufficiently high in protein to be designated a protein concentrate (i.e., 70% protein on dry weight basis). As improved UF membranes become available, it is expected the protein content of each product will be enriched by a

TABLE IV. YIELDS OF PRODUCTS FROM MEMBRANE PROCESSING COTTONSEED WHEYS FROM PROCESSES B, C, AND F[1]

Type Whey Processed	Lb. Whey / Lb. Flour	Lb. UF Prod. / Ton Flour	Lb. RO Prod. / Ton Flour	Lb. UF Prod. / 1,000 Lb. Whey	Lb. RO Prod. / 1,000 Lb. Whey	Lb. UF Prod. / Lb. Protein Isolate
Process B						
Whey	16.6	231.58	343.92	6.98	10.36	0.28
Process C						
NSP-Whey	12.9	247.4	192.4	9.59	7.46	
SP-Whey	20.3	283.3	65.8	6.98	2.55	0.74
Process F						
Major whey	12.3	313.0	882.4	12.72	35.87	
Minor whey	4.03	79.6	78.0	9.88	9.68	0.63

[1]All dry products calculated to 6.9% m.b.

TABLE V. ANALYTICAL DATA ON DRY PRODUCTS FROM MEMBRANE PROCESSING GLANDLESS COTTONSEED WHEYS
(% dry wt. basis)

Product Identification[1]	Moisture %	Ash	Oil	Gossypol		Nitrogen		Protein	Phosphorus		Carbo-hydrates
				Total	Free	Total	Nonprotein	(N × 6.25)	Total	Inorg.	
UF B	6.1	8.4	0.10	0.02	0.00	11.6	3.6	72.3	2.30	2.08	23.3
RO B	13.2	34.3	0.06	...	...	2.3	2.3	13.9	11.0	10.5	46.6
UF C-NSP	6.7	6.2	0.60	0.02	0.01	10.3	2.6	64.3	1.49	1.17	33.8
UF C-SP	5.5	6.5	3.4	0.02	0.01	12.1	1.4	75.9	1.47	0.74	8.3
RO C	9.3	20.3	0.15	...	...	3.9	3.9	24.2	5.36	4.83	36.1
UF F-Major	7.2	10.9	0.30	0.09	0.02	12.5	2.8	78.1	1.00	0.90	13.6
UF F-Minor	2.1	18.4	0.10	0.02	0.02	11.2	1.7	70.0	0.21	0.20	14.8
RO F	10.0	48.3	0.05	...	...	2.5	2.5	15.6	2.26	2.04	19.5

[1]Products designated by membrane type, isolation process, and whey type.

TABLE VI. AMINO ACID ANALYSES OF PRODUCTS FROM MEMBRANE PROCESSING OF COTTONSEED WHEYS (g. per 16 g. N)

Amino Acids	Process B			Process C					Process F		
	Whey	UF-B	RO-B	C-NSP whey	C-SP whey	UF C-NSP	UF C-SP	RO-C	Major whey	UF-F major	UF-F minor
Lysine (total)	5.5	6.1	2.3	6.0	4.2	6.9	5.9	6.6	4.9	6.5	6.0
(avail.)	5.2	6.1	2.3	5.6	4.1	6.4	5.6	6.6	3.9	5.8	6.0
Histidine	2.0	2.2	0.9	1.8	2.3	1.7	2.7	1.4	2.2	2.7	2.3
Arginine	12.7	12.8	11.5	12.4	13.0	12.3	10.6	6.9	13.5	14.4	12.8
Tryptophan	1.0	1.3	1.3	1.2	1.2	1.3	1.6	1.2	1.3	1.6	1.6
Cystine	5.0	5.7	2.4	4.4	3.0	5.2	2.3	3.6	3.8	6.1	4.5
Aspartic acid	7.1	7.3	5.6	7.0	6.7	6.6	8.4	4.8	6.7	7.3	8.0
Threonine	2.5	2.5	1.3	2.5	2.8	2.8	3.9	1.7	2.1	2.4	2.7
Serine	2.4	2.5	1.5	1.2	3.2	2.4	4.1	1.6	2.7	3.4	3.8
Glutamic acid	24.3	26.3	12.7	17.3	21.5	23.7	19.3	17.7	21.8	29.6	26.4
Proline	3.0	3.1	1.6	4.1	3.1	2.9	3.9	2.5	2.3	3.8	3.5
Glycine	3.6	3.4	2.9	3.3	4.2	3.1	4.6	3.3	3.4	4.1	4.1
Alanine	2.6	2.4	2.3	2.9	3.1	2.9	4.6	2.3	2.2	2.4	3.1
Valine	1.7	1.9	1.3	2.1	2.9	1.9	4.5	1.4	2.1	1.9	2.6
Methionine	0.9	1.4	0.8	0.8	1.8	1.2	2.2	1.0	1.3	1.6	1.2
Isoleucine	1.1	1.2	0.8	1.3	2.1	1.1	3.4	1.0	1.3	1.5	1.8
Leucine	2.1	2.3	1.6	2.5	4.4	2.2	6.5	1.7	2.7	2.7	3.5
Tyrosine	2.7	3.1	1.4	2.5	2.4	2.8	3.6	2.1	2.3	3.0	2.9
Phenylalanine	2.0	1.9	1.5	2.0	3.1	1.9	4.7	1.6	2.2	2.0	3.3
% Amino acid N	78.2	81.4	60.0	73.8	80.8	78.3	87.5	56.9	77.0	92.2	88.1

more complete separation of salts and sugars from the protein. Utilization tests on some of these UF products have previously been reported (5). No attempts have as yet been made to find uses for the RO products.

Table VI contains amino acid analyses on unfractionated whey solids from four of the five wheys and on UF and RO products from all five wheys. The quality of protein in the products was increased slightly by ultrafiltration as reflected by the increase in percentage amino acid nitrogen. Also ultrafiltration increased several amino acids above the level of their occurrence in the parent flour (5). For example, lysine and cystine were increased from 4.0 and 2.4 g. per 16 g. N in the original flour (not shown) to 6.1 and 5.7, respectively, in UF B product.

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