Polymeric Membranes for the Separation of the Proteins in Liquids for Alimentary Use

DANIELA RATA*, LILIANA VIORICA PASARE, BUJOR GABRIEL ALBU, MARIN RADU
The Research Centre for Macromolecular Materials and Membranes, 202B Splaiul Independentei, 060023, Bucharest, Romania

This paper presents the preparation by phase inversion of polysulfone and cellulose acetate ultrafiltration membranes (UF1-UF36) with various amounts of PEG additives (1000, 2000, 4000 Da), designed for the whey protein separation. To establish optimal operating conditions for membrane separation and the membrane with the best characteristics for the ultrafiltration of whey, we determined the flow variation of the two types of whey (pH=6.21, pH=4.35) with different parameters: whey temperature, operating pressure, protein retention and clogging phenomenon. All the results indicated that at the same protein separation efficiency (MWCO), the polysulfone membranes present higher flows than cellulose acetate membranes and their use in the acid whey ultrafiltration process lead to a whey protein concentrate with a protein content about 5 times higher than the initial whey content (6.4 kg/m^3). The best protein retention 98.73% was obtained for UF18 membrane (20% PS, 2 % PEG 4000), with optimal flow rate (23.9·10^{-6} m^3/m^{2}s) for acid whey, operating at 50°C and 10^6 Pa.

Keywords: membranes, ultrafiltration, proteins, recovery

The obtaining and use of polymeric membranes in phase separation processes is a current practice even at industrial scale, with increasing evolution in the efficiency and in the economic results. The advantage of using membranes is their selective permeation.

Structural requirements and membrane performances are determined by the type of the media (*i.e.* nature and composition) subjected to the separation, the properties of the system components, the liquid volume considered to be processed. From the structural point of view, the polymeric membranes may be divided in symmetrical, asymmetrical and composite membranes.

In this paper the potential applications of polymeric membranes for whey protein separation are examined. The membrane processes are well applied to the separation and concentration of the whey proteins, since they occur at ambient temperature without the denaturation of bioactive substances or changes in organoleptic properties. Whey membrane filtration provides fractional separation in products with specific uses [1].

Since about half of the processed milk is transformed in whey, the main by-product containing proteins with the highest known biological value, its revaluation by membrane processes is imposed as a requirement for the protein recovery [2].

Ultrafiltration (UF) is the membrane process with the highest applicability among the whey protein processing techniques because the ultrafiltration step yields a retentate having the butterfat and non-fat solids content required for cheese manufacture [3-5].

The main proteins from whey are the serum albumin (8%) with the molecular weight M = 67.000 Da and average hydrodynamic radius 3.5 nm; β-lactoglobulin-dimer (65%), with the molecular weight M=36.000 Da and average hydrodynamic radius 2.6 nm and α-lactalbumin (25%) respectively, with the molecular weight M=14.200 Da average hydrodynamic radius 3 nm. The protein concentrate obtained from the whey contains 18 essential amino acids, important for the living bodies [6,7].

Despite continuing efforts to find uses for the whey, in Romania only about 50% of the whey is used primarily for animal feed or human food while the rest is disposed as a waste with negative impact on the environment [8-10].

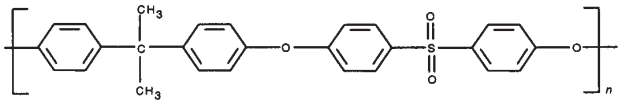
Taking into account the above-mentioned aspects, our work is focused on the identification of new membranes that might simultaneously fractionate, purify and concentrate whey components, thus enhancing their utilization and reducing the pollution problem.

Ultrafiltration polysulfone (**UF1-UF18**) and cellulose acetate membranes (**UF19-UF36**) with PEG1000, 2000, 4000 as additives have been obtained and characterized. These membranes have hydrodynamic (permeate flow) and performance (protein retention) features superior to those established in this field (Millipore, Pall Gelman and Desal).

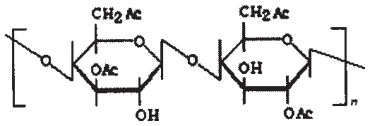
Experimentsl part

Materials and methods

Polysulfone (PS) of the ULTRASON S 3010 (BASF) type, with base unit formula:



Cellulose acetate (AcC) with 39.8% acetyl groups (ALDRICH CHEMICAL Co.), with the generic formula:



Polyethylene glycol – PEG with the following molecular weights 1000, 2000, 4000 Da (Fluka), N-methyl-pyrrolidone - NMP (Merck) and demineralised water (conductivity 5·10^{-4} S/m), MEMBRASEP®, microfiltration membrane with average pore size 25·10^{-8} m (0.25μm), The Research Centre for Macromolecular Materials and Membranes trademark.

According to the cheese manufacturing process there are two types of whey (table 1):the *Molecular Weight Cut-Off - MWCO* value (defined as the molecular weight of a hypothetical substance which is retained in a proportion of at least 90% by the membrane) was determined by

* *Tel.: (+40) 0213162816*

Table 1
WHEY COMPOSITION

Whey type	pH	Dried substance [kg/m^3]	Fats [kg/m^3]	Proteins [kg/m^3]	Other soluble compounds [kg/m^3]
Sweet whey	6.21	68.5	2.9	5.8	59.8
Acid whey	4.35	69.7	1.1	6.4	62.2

Table 2
KNOWN MOLECULAR WEIGHT SUBSTANCES FOR *MWCO* DETERMINATION OF ULTRAFILTRATION MEMBRANES **UF1-UF36**

Substance	Molecular weight (Da)	Maximum absorption wavelength, (λ_{max}, nm)
Cytochrome C	12400	400, 520
Lysozym	14000	292
α- chemotripsin	25000	284
β-lactoglobulin	35000	210-280
Egg Albumin	44000	280
Hemoglobin	64000	275
Bovine serum albumin	66500	210, 278

spectrophotometric methods [11] using substances with known molecular weight (table 2).

The UV–Vis spectra in solution for these substances (Table 2) have been recorded with a GBC– 918 Scientific Equipment Pty Ltd.–Australia spectrophotometer, in quartz cells with 10 mm light path.

The retention of the substances used for *MWCO* determination and the protein retention were calculated with the formula:

$$R = (C_f - C_p)/C_f = 1 - C_p/C_f \quad (1)$$

where:

C_f - the solute concentration in the feed fluid,

C_p - the solute concentration in the permeate.

The whey fat content was determined according to SR EN ISO 1211/ 2003.

The protein analysis was performed by Kjeldahl method according to SR EN ISO 8968-5:2002.

Preparation and characterization of the ultrafiltration membranes

Ultrafiltration membranes have been prepared from polysulfone (**UF1-UF18**) and from cellulose acetate (**UF19-UF36**) polymeric solutions in NMP with PEG1000, 2000, 4000 (1%wt or 2% wt content) as additives (tables 3, 4).

The ultrafiltration membranes have been obtained by phase inversion, which consists in casting the polymeric solutions as thin films onto a glass plate, by using a calibrated device with a slit of 0.2 mm ("doctor Blade") [11-13]. Once cast, the solvent is partially evaporated prior to submersion of the polymeric film into a deionised water bath ($5 \cdot 10^{-4}$ S/m). The compositions of the polymeric solutions and the characteristics of the prepared membranes are presented in tables 3 and 4.

The polymers (PS and AcC) for the preparation of the membranes have been chosen on the basis of their good solubility in usual solvents and their good chemical

Table 3
COMPOSITION OF THE PS POLYMER SOLUTIONS AND MEMBRANES CHARACTERISTICS (**UF1-UF18**)

Membrane	Polymer concentration [%]	Type of admixture	Admixture concentration [%]	MWCO [Da]	Distilled water normalized flux* $J \cdot 10^6$ [m^3/m^{2}s]
UF1	15	PEG1000	1	66500	81.3
UF2	15	PEG1000	2	66500	82.6
UF3	15	PEG2000	1	66500	83.3
UF4	15	PEG2000	2	66500	84.5
UF5	15	PEG4000	1	66500	87.6
UF6	15	PEG4000	2	66500	88.7
UF7	18	PEG1000	1	25000	53.3
UF8	18	PEG1000	2	25000	55.1
UF9	18	PEG2000	1	35000	56.7
UF10	18	PEG2000	2	35000	57.8
UF11	18	PEG4000	1	44000	61.2
UF12	18	PEG4000	2	44000	62.8
UF13	20	PEG1000	1	12400	35.2
UF14	20	PEG1000	2	14000	36.9
UF15	20	PEG2000	1	12400	38.2
UF16	20	PEG2000	2	14000	39.1
UF17	20	PEG4000	1	14000	42.5
UF18	20	PEG4000	2	12400	45.8

*$J = \frac{V}{t \cdot s}$, *V – volume (m^3), t – time (s), s – membrane surface (m^2).*

Table 4
COMPOSITION OF THE AcC POLYMER SOLUTIONS AND MEMBRANES CHARACTERISTICS (**UF19-UF36**)

Membrane	Polymer concentration [%]	Type of admixture	Admixture concentration [%]	MWCO [Da]	Distilled water normalized flux* $J \cdot 10^6$ [m^3/m^2s]
UF19	15	PEG1000	1	66500	61.8
UF20	15	PEG1000	2	66500	63.4
UF21	15	PEG2000	1	66500	63.7
UF22	15	PEG2000	2	66500	65.2
UF23	15	PEG4000	1	66500	69.2
UF24	15	PEG4000	2	66500	70.8
UF25	18	PEG1000	1	25000	43.1
UF26	18	PEG1000	2	25000	45.5
UF27	18	PEG2000	1	35000	44.9
UF28	18	PEG2000	2	35000	45.6
UF29	18	PEG4000	1	35000	47.7
UF30	18	PEG4000	2	35000	48.3
UF31	20	PEG1000	1	12400	32.5
UF32	20	PEG1000	2	12400	33.9
UF33	20	PEG2000	1	12400	33.6
UF34	20	PEG2000	2	12400	34.2
UF35	20	PEG4000	1	12400	36.4
UF36	20	PEG4000	2	12400	37.9

* $J = \frac{V}{t \cdot s}$, *V – volume (m^3), t – time (s), s – membrane surface (m^2).*

resistance (*i.e.* oxidation); they also have a good thermal stability, characteristic required for the use of membranes in food where the sterilisation is compulsory. The PEG additives were used for the adjustment of membrane porosity and pore size control. Although these additives are soluble in water and are removed from membranes during the coagulation step, their concentration was maintained at 1-2%wt in order to achieve both an appropriate viscosity for the casting of the polymer solutions and a good mechanical strength of the membranes. The membranes are conditioned in a water and glycerine bath to preserve their structural and performance characteristics [14-17].

The membranes have been characterized both hydrodynamically (the normalized flow of distilled water at standard pressure of 10^6 Pa for ultrafiltration) and for evaluation of separation efficiency using a CELFA Membran-Systeme filtration module (fig. 1). For each membrane has been carried out an experiment leading to the obtaining of a whey protein concentrate.

The membrane is inserted in compartment (1), and a volume of $5x10^{-4}$ m^3 whey is introduced in the feed tank (2). The gear pump (3) and the thermostat unit (4) are started simultaneously and the whey is recirculated to achieve the predetermined temperature (50°C) at normal pressure ($1.013x10^5$Pa). After reaching the operating temperature the process parameters are adjusted: the operating pressure indicated by the gauge manometer (9) at the predetermined value (10^6Pa) by the admission of nitrogen from cylinder (12) and the pressure gauge (10) and also the permeate flow by reflux valve (6).

The process continues until the required concentration ratio (1/5) is reached and about $4x10^{-4}m^3$ permeate is collected. The nitrogen inlet closes while the pump (3) and the thermostat (4) stop. The entire filtration module is depressurized by gradually opening the air valve (5). Protein concentrate is collected by opening the valve (7) and then the experimental setup is washed for another experiment.

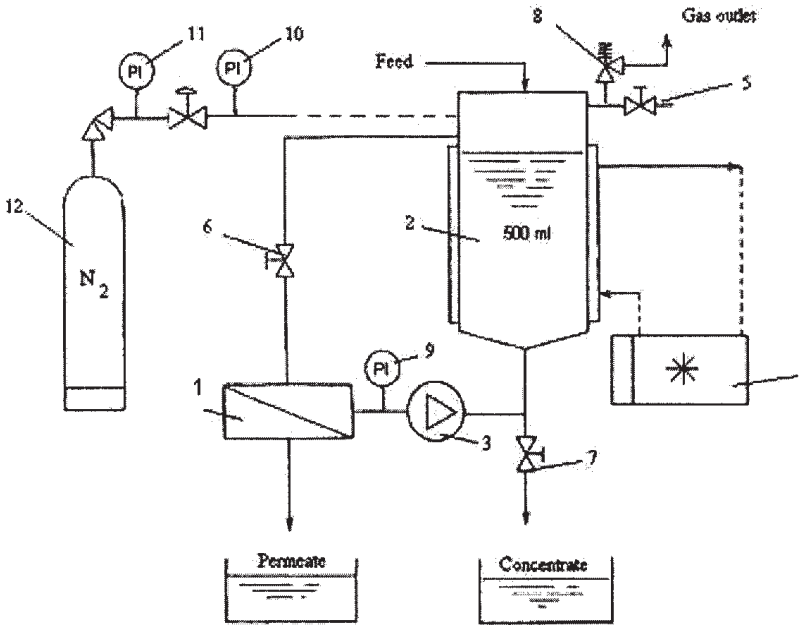


Fig. 1. CELFA Membran-Systeme Laboratory Unit P28 experimental setup
1-membrane cell, 2- feed tank, 3- gear pump, 4- thermostat unit, 5- air valve, 6- reflux valve, 7- concentrate valve, 8- safety valve, 9-11- pressure gauges, 12 –nitrogen gas cylinder

Table 5
COMPOSITION OF THE MICROFILTRATION PERMEATES

Whey type	pH	SU [kg/m^3]	Fats [kg/m^3]	Proteins [kg/m^3]	Other soluble compounds [kg/m^3]
Sweet whey	6.21	65.4	0.0001	5.67	59.8
Acid whey	4.35	68.4	-	6.30	62.2

The whey has been microfiltered on the MEMBRASEP® membrane to remove the mechanical impurities and the fats and to increase the life span of the ultrafiltration membranes. Permeates are used as feeding liquid for the ultrafiltration process. The composition of the microfiltration permeates is different from that of the initial whey in the sense that the fats are absent and the remaining components are significantly reduced (table 5).

Results and discussions

The results of the membrane characterization assays lead to the following observations:

- the normalized flow of distilled water and the *MWCO* decrease directly proportional with the increasing polymer concentration of the solutions used in the preparation of the membranes (tables 3 and 4);
- at the same *MWCO,* the polysulfone membranes (**UF1-UF18**) present higher water flows across the membrane than cellulose acetate membranes (**UF19-UF36**);
- the membranes (**UF13-UF18** and **UF31-UF36**), whose *MWCO* values are in the 12400-14000 Da range, are suitable for the separation of whey proteins since the lowest molecular weight whey protein is the β-lactalbumin (M = 14200 Da).

Based on these observations, the polysulfone membranes **UF13-UF18** having, at the same *MWCO*, higher flows than cellulose acetate membranes (**UF31-UF36**) have been selected for subsequent applications in ultrafiltration processes.

In order to establish optimal operating conditions and the membrane having the best characteristics for the ultrafiltration of whey we determined the flow variation of the two types of whey with different parameters: a) whey temperature, b) operating pressure, c) protein retention, d) membrane type – polysulfone with 1 or 2% wt content of different additives PEG1000, 2000, 4000 Da and e) the clogging phenomenon. The experimental results are presented in figures 2–7.

From the plots of the flow *versus* temperature (fig. 2 and 3), one may conclude that regardless the nature of the whey (sweet whey – *p*H 6.21 or acid whey – *p*H 4.35), the whey flows reach a maximum at temperature of 50°C, then decrease for temperatures higher than 60°C. This behaviour might be attributed to the protein denaturation process as the temperature rises and to the fact that the macromolecular compounds clog the membranes. Given the fact that the optimal temperature for the separation process is 50°C, the subsequent tests have been carried out at this temperature.

For both types of whey, the plots of the variation of the whey flow with the pressure have the same general aspect (fig. 4-5) with the slope of the curve being less influenced by the whey nature. The flow increases with the pressure up to 10^6Pa followed by a linear variation in 10^6–$1.5 \cdot 10^6$Pa pressure range when the compaction of the membrane structure occurs. The best flows for the both types of whey are obtained for **UF18** membrane at 10^6Pa, the pressure appropriate for ultrafiltration process, with optimal flow value for acid whey.

The analysis of protein retention data for each membrane (table 6) for both types of whey shows that

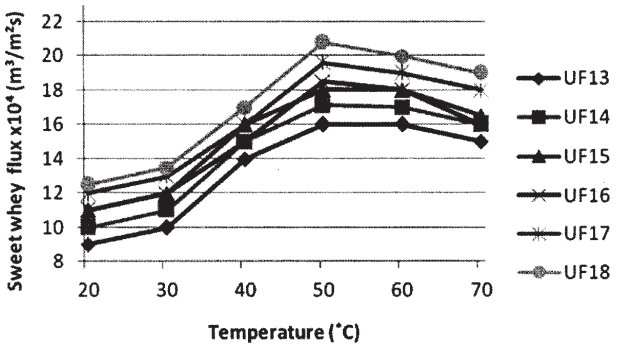


Fig. 2. Plot of sweet whey flow *versus* temperature

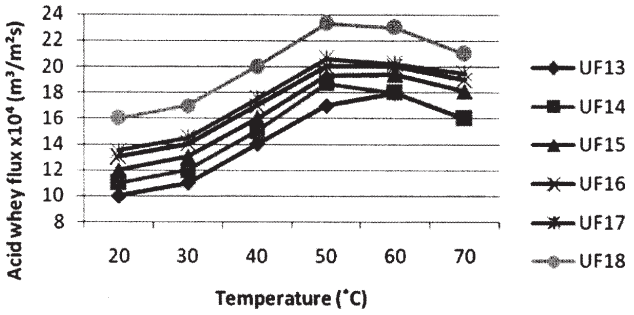


Fig. 3. Plot of acid whey flow *versus* temperature

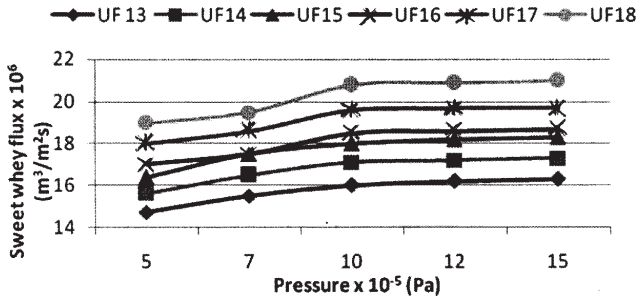


Fig. 4. Plot of sweet whey flow *versus* operating pressure

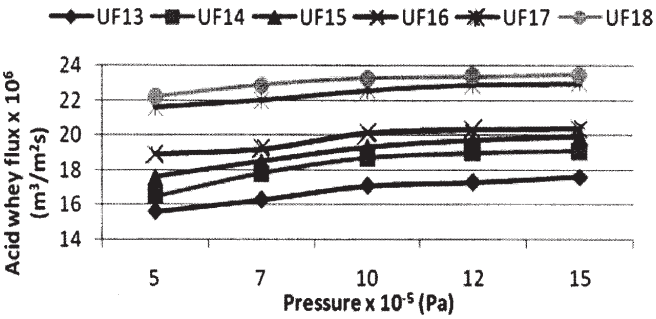


Fig. 5. Plot of acid whey flow *versus* operating pressure

protein retention on membranes exceeds 94% in all cases and is not significantly dependent on the whey nature (*p*H = 6.21 or 4.35).

The higher flows have been obtained for the acid whey irrespective of the membrane composition (polysulfone with 1% or 2% wt content of different additives PEG1000, 2000, 4000, table 3) with the best flows for the **UF16-UF18** membranes (fig. 6).

Table 6
PROTEIN RETENTION RESULTS ON **UF13-UF18** MEMBRANES

Membrane	Acid whey retention (%)	Sweet whey retention (%)
UF13	96.4	95.2
UF14	96.1	95.1
UF15	96.5	95.0
UF16	96.2	95.2
UF17	96.3	95.3
UF18	96.6	95.2

Table 7
PROTEIN CONCENTRATION RESULTS ON THE **UF18** MEMBRANE

Whey protein concentration [Kg/m^3]	Permeate flow $\cdot 10^6$ [m^3/m^{2}s]	Pressure $\cdot 10^{-5}$ [Pa]	Protein concentration in permeate$\cdot 10^4$ [Kg/m^3]	Protein concentration in retentate$\cdot 10^4$ [Kg/m^3]	Efficiency of protein recovery [%]
6.3	23.90	10.0	0.08	36.67	98.73

These results and the permeate flow – protein retention correlation established that the **UF18** membrane is the best membrane for whey ultrafiltration (operating temperature-50°C and pressure-10^6Pa).

As membrane clogging is an essential aspect of ultrafiltration in whey processing (it reduces membrane flow and increases the operating costs by introducing the membrane cleaning phases), the phenomenon has been studied by the monitoring the membrane permeate flow variation in time (fig. 7). The clogging phenomenon occurs as a result of the existence of an absorption layer by physical and chemical interactions between the membrane and the protein components in whey. The effect of the **UF18** membrane clogging on the permeate flow has been observed for 96 h (50°C, 10^6Pa).

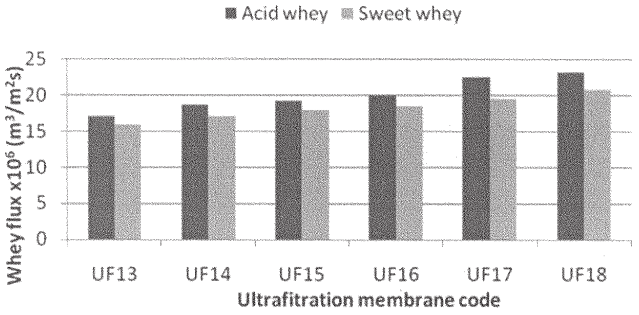


Fig. 6. The variation of the whey flow with the whey and the membrane type

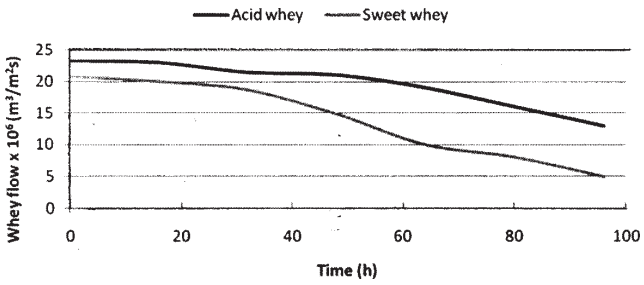


Fig. 7. Effect of the **UF18** membrane clogging on the permeate flow

The static and dynamic clogging lead to the decreasing of the whey flow in time (fig. 7) and this phenomenon is more pronounced for the sweet whey, where the permeate flow drastically decreases after about 30 h. The clogging is slower in the case of the acid whey, the phenomenon being visible only after about 55 h.

All the results pointed out that for ultrafiltration process efficiency the whey needs to be acidified. At lower *p*H, the whey is better processed for the protein concentration and at the same time the membrane lifespan is increased. To reduce the negative effects of the clogging, the membranes are washed regularly with an alkaline solution in counterflow and by setting a tangential flow of the whey during its processing.

Consequently, in order to obtain whey protein concentrates it is recommended to process the acid whey through **UF18** membrane, at a pressure of 10^6Pa and a temperature of 50°C, when the maximum membrane flow is of 23.9×10^{-6} m^3/m^{2}s and the protein retention is above 98,73% (table 7). For a protein concentration experiment carried out in these conditions, **UF18** membrane leads to a protein concentrate (retentate) with a protein content about 5 times higher than the initial one while the protein losses in the permeate are below 1.3%.

Conclusions

The ultrafiltration membranes from polysulfone (**UF1-UF18)** and cellulose acetate (**UF19-UF36**) have been prepared by phase inversion, characterized hydrodynamically (the normalized flow of distilled water at standard pressure of 10^6Pa for ultrafiltration) and for evaluation of protein separation efficiency (*MWCO*).

The membranes (**UF13-UF18** and **UF31-UF36**), whose *MWCO* values are in the 12400-14000 Da range, are suitable for the separation of whey proteins since the lowest molecular weight whey protein is the α-lactalbumin (M = 14200 Da). At the same *MWCO*, polysulfone membranes have higher flows than cellulose acetate membranes.

To establish optimal operating conditions and the membrane having the best characteristics for the ultrafiltration of whey we determined the flow variation of the two types of whey with different parameters: whey temperature, operating pressure, protein retention and clogging phenomenon.

The best protein retention 98,73% are obtained for **UF18** membrane, with optimal flow value (23.9×10^{-6} m^3/m^{2}s) for acid whey operating at temperature of 50°C and at a pressure of 10^6Pa. The protein concentrate (retentate) has a protein content about 5 times higher than the initial one while the protein losses in the permeate are below 1.3%.

References

1.SCHIEK, B., Dairy notes, **16-08,** Dairy Institute of California, Sacramento, 2008
2.BELL, S J., J. Med Food, **3,** 2000, p.1
3.SANGITA, B., CHIRANJIB, B., DATTA, S., J Membr. Sci., **275**, nr.1-2, 2006, p. 141

4.MARSHALL, A.D., MUNRO, P.A., TRAGARDH, G., Desalination, **91**,1993, p. 65
5.RENNER, E., EL-SALAM, M.H.A., Application of Ultrafiltration in the Dairy Industry, Elsevier Applied Science, London, 1991, p. 78
6.TUINIER, R., DHONT, J. K. G., . DE KRUIF, C. G., Langmuir**, 16,** nr. 4, 2000, p 1497
7.KRISTIANSEN ,K. R., OTTE, J., IPSEN, R., I. Dairy J., **8**, nr. 2,1998, p. 113
8.NODA, T., IWAKIRI, FUJIMOTO, K., RHOADS, C. A., AW, T. Y, Cell Proliferation, **35**, nr. 2, 2002 , p. 117
9.GAIN, EU-25,Dairy and Products, EU market for Whey derivatives 2006, **E36135,** Unscheduled Report Brussels USEU
10.TONG, LM, SASAKI, S, MCCLEMENTS, DJ, DECKER, EA, J Agric Food Chem., **48,** 2000, p.1473
11.BAKER, R. W., Membrane Technology and Application, 2nd ed., John Wiley & Sons, Ltd., The Atrium, Southern Gate, Chichester, West Sussex,England, 2004, p.237
12.CHMIEL, H., MAVROV, V., BELIERES, E.,Filtration-Separation, **37,** nr. 3, 2000, p.24
13.VIEZURE, I., PASARE, L., RATA, D., DUMITRU, F., GURAN, C., Rev. Chim. (Bucuresti)**, 57**, no. 2, 2006, p. 170
14.VIEZURE, I., PASARE, L., RADU, M., DUMITRU, F., GURAN, C., Rev. Chim. (Bucuresti), **57**, no. 9, 2006, p. 931
15.LEHR, C., PASARE, L., NITA, O., POPESCU, G., Rev. Chim. (Bucuresti), **55,** no.12, 2004, p. 939
16.PASARE, L., MARUTA, C., RATA, D., VIEZURE, I, ALBU, B.G., RADU, M,. Rev. Chim. (Bucuresti), **54**, no.9, 2003, p. 763
GARGANCIUC D., BĂTRÎNESCU GHE., POPA O., OLTEANU M., Mat. Plast., **45**, no. 2,2008, p.29

Manuscript received: 6.04.2009