Review - Pregledni rad

UDK: 637.072

Membrane processes in production of functional whey components

Arzu Akpinar-Bayizit*, Tulay Ozcan, Lutfiye Yilmaz-Ersan

Uludag University, Department of Food Engineering 16059 Gorukle, Bursa, Turkey

> Received - Prispjelo: 02.10.2009. Accepted - Prihvaćeno: 15.11.2009.

Summary

In recent years, whey has been recognised as a major source of nutritional and functional ingredients for the food industry. Commercial whey products include various powders, whey protein concentrates and isolates, and fractionated proteins, such as α -lactalbumin and β -lactoglobulin. The increased interest in separation and fractionation of whey proteins arises from the differences in their functional, biological and nutritional properties. In response to concerns about environmental aspects, research has been focused on membrane filtration technology, which provides exciting new opportunities for large-scale protein and lactose fractionation. Membrane separation is such technique in which particles are separated according to their molecular size. The types of membrane processing techniques are ultrafiltration, microfiltration, reverse osmosis, pervaporation, electrodialysis and nanofiltration. A higher purification of whey proteins is possible by combining membrane separation with ion-exchange. This paper provides an overview of types and applications of membrane separation techniques in whey processing and production of whey-based functional compounds.

Key words: whey, membrane processes, functional compounds

Introduction

Whey, a valuable by-product of cheese manufacture, is the liquid fraction drained from the curd. Recently, the biological properties of individual whey constituents, such as α -lactalbumin (α -La) and β -lactoglobulin (β -Lg), lactoperoxidase (LP), lactoferrin (LF), glycomacropeptide (GMP) and immunoglobulins (IgGs), have become a focus of commercial interest as potential ingredients of so-called functional or health-promoting foods.

They are known to be involved in antioxidant activity, anticarcinogenic effects, immunomodulation, passive immunity, anti-microbial effects, binding of toxins, promotion of cell growth, platelet binding, anti-inflammatory and anti-hypertensive actions (Kinsella and Whitehead, 1989; Madureira et al., 2007; Smithers, 2008; Chicón et al., 2009; McClements et al., 2009).

Enthusiasm for seeking benefits from diet and nutritional supplements that help maintain physical well being due to active lifestyles, makes people familiar with whey proteins and amino acid supplements. Recent studies demonstrate directly that whey proteins and their constituent amino acids efficiently promote protein synthesis (Ha and Zemel, 2003).

Processing of cheese whey was the first successful commercial application with several research initiatives underway to find uses of whey-waste. Since whey disposal is costly and problematic for cheese manufacturers; the focus is on techniques to

^{*}Corresponding author/Dopisni autor: Phone/Tel: +90 224 29 41 496; E-mail: abayizit@uludag.edu.tr

convert this waste product into valuable functional components (Pouliot, 2008).

Filtration is the separation of two or more components from a fluid stream. The filtration membrane can be described as an interphase that acts as a selective barrier, allowing the flow of certain molecular and ionic components and retaining others present in a liquid or vapour mixture. In membrane separation, a similar technique to traditional filtration, particles are separated on the basis of their molecular size and shape with the use of pressure and specially-designed semi-permeable membranes. The most common membrane processes are ultrafiltration (UF), microfiltration (MF), reverse osmosis (hyperfiltration) (RO/HF), pervaporation, nanofiltration (NF), electrodialysis (ED) (Zeman and Zydney, 1996; Brans et al., 2004; Rektor and Vatai, 2004; Saxena et al., 2009).

The rapid developments in membrane filtration techniques in the 1970's provided new possibilities for whey proteins manufacture. Since then, whey protein concentrates (WPC) and isolates (WPI) with various properties have been produced as food ingredients (Van Reis and Zydney, 2007). The current research and development efforts in membrane processes are directed towards improvements in membrane modules and systems to meet the requirements of food industry with accordance to health benefits (Saboya and Maubois, 2000; Baruah et al., 2005; Yorgun et al., 2008; Saufi and Fee, 2009). The objective of the current paper is to focus on membrane techniques used in production of functional whey components.

Membrane process applications in dairy industry

The commercial membrane applications in the dairy industry can be classified into four main areas, i) alternatives to some unit operations (i.e. centrifugation, evaporation, debacterization and demineralization), ii) resolving separation issues (i.e. defatting of whey, protein recovery and separation, milk fat globule fractionation and spore removal), iii) creating new dairy products (i.e. UF cheeses, whey-based beverages and textured milk products, functional whey components), and iv) improvement of cheese yield and product consistency (Atkinson, 2003).

Every 100 kg of milk used in cheese manufacture results in 10-20 kg of cheese and 80-90 kg of whey. Whey consists of 0.6 % protein, 0.1 % fat, 5.0 % lactose, 0.003 % casein, 0.18 % non-protein nitrogen (NPN), 0.6 % ash and 6.03 % total solids (Kosikowski and Mistry, 1997). The major proteins present in whey include α -lactalbumin, β -lactoglobulin, immunoglobulin, bovine serum albumin, and glycomacropeptide. Minor, but commercially important proteins are lactoferrin and lactoperoxidase (Doultani et al., 2004). Even though individual components of whey (proteins and lactose) have known applications, whey itself is difficult to dispose of or utilize, due to its unfavorable lactoseto-protein ratio and high biological oxygen demand of 30 000-50 000 ppm (Kinsella and Whitehead, 1989; Smith, 2001).

Whey processing is one of the most successful industrial membrane applications. For reasons of simplicity, urgency and the economics of disposal problem solution, UF of whey was the first application of membrane fractionation to reach a full commercial scale. The membranes used should be high yielding, resistant to physical, chemical and microbiological agents, unaffected by cleaning and disinfection materials (Yorgun et al., 2008; Bhushan and Etzel, 2009; Cuartas-Uribe et al., 2009). Figure 1 shows a general scheme of possible membrane applications in whey treatment (Cheryan, 1998).

There are two kinds of whey: sweet whey (resulting from cheeses that are produced by addition of rennet, and thus the pH is 5.8-6.6) and acid whey (which results from cheeses made by acidification, with a pH of <5.0) (Zadow, 1994). The latter generally has twice the amount of calcium phosphate and more lactic acid. Calcium phosphate is more soluble at low pH and low temperature. Thus the operating conditions for membrane filtration, such as temperature and pH, should be selected with accordance to the behavior of calcium salts, in addition to considerations of viscosity, protein denaturation and microbial growth (Pacheco et al., 2002; Tolkach and Kulozik, 2005).

Applications of membranes in whey processing include a) concentration of whey 3 folds (24 %) with RO and NF prior to evaporation and drying, b) manufacture of WPI (90 %), c) production of WPC (35- 80 % protein), d) converting the lactose to higher-value products by fermentation (e.g., ethanol or lactic acid) or by enzyme hydrolysis in continuous membrane reactors, e) fractionation of

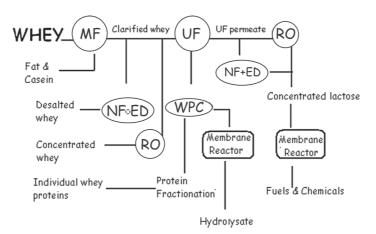


Fig. 1: Membrane applications used in whey processing (Cheryan, 1998) Slika 1: Primjena membranskih procesa u preradi sirutke (Cheryan, 1998.)

whey to value-added nutraceuticals, f) MF of whey as a pre-treatment for UF, and g) concentration and demineralization of whey and UF permeate with NF (Cheryan, 1998; Atra et al., 2005).

Even though it is not commonly applied for whey fractionation and purification, pervaporation is a membrane process used to separate liquid mixtures. In pervaporation process, the feed liquid contacts one side of a membrane, that selectively permits flow of one of the feed components. The permeate, enriched with this component, is removed as a vapor from the other side of the membrane. The driving force for the process is the difference in vapor pressure between the feed liquid and the permeate vapour (Baker et al., 1997). Ion-exchange process is used for general fractionation of particules by liquid chromatographic techniques, and when combined with commercial membrane systems a high-purification of whey proteins can be achieved. In the most commonly used ion-exchange process, the whey is acidified to the point that most of the proteins have a net positive charge. The positively-charged proteins bind to the resin, allowing everything else to pass through. After the unbound materials (mostly fat and lactose) are washed out of the resin bed, the pH is increased to release the bound proteins, which are then ultrafiltered (Chiu and Etzel, 1997; Gerberdingand and Byers, 1998; Greiter et al., 2002).

By UF, liquid whey is simultaneously fractionated, purified, and concentrated to whey protein concentrate (WPC). UF is driven by a pressure gradient, using 275.9 kPa and temperatures of 50-60 °C with polysulfone membranes with a membrane pore size between 10^{-1} - 10^{-2} µm. There are four primary configurations of UF membrane modules: tubular, hollow-fiber (HF), spiral-wound and flat plate. Each configuration has their inherent strengths and weaknesses, and varies in their industrial and commercial applications. Choosing the right module configuration is important to the overall effectiveness of the UF process (Cheryan, 1998; Zydney, 1998).

WPCs produced by UF have a protein content ranging from 34 to less than 90 %. When the protein concentration exceeds 90 % the product is known as whey protein isolate (WPI). To increase the protein concentration, a modification of the process, known as diafiltration, can be employed to further decrease the lactose and mineral content. As the lactose content is decreased, the WPCs can be used to enrich the protein content of a food without increasing the browning associated with lactose. The high-protein WPCs also become more acceptable to the large numbers of people who cannot effectively digest lactose in their diet (DaCosta et al., 1993; Bhushan and Etzel, 2009).

The major application of MF in whey processing is as a pretreatment for UF, and designates a process similar to UF but with even larger membrane poresize allowing particles in the range of 0.2-2 μ m to pass through (Zeman and Zydney, 1996). Whey usually contains small quantities of fat and casein, and since centrifugal separation does not completely remove them, MF is used to reduce fat-to-protein

284

ratios to 0.001-0.003 (Saboya and Maubois, 2000; Baruah et al., 2005; Nelson and Barbano, 2005). In addition, some of the precipitated salts may be removed by MF, and there is a considerable reduction in microbial load (Van der Horst and Hanemaarjer, 1990; Maubois, 1997).

Another pressure-driven membrane process is reverse osmosis (RO) in which membrane pore size is very small allowing only small amounts of very low molecular weight solutes to pass through (Del Re et al., 1998; Balannec et al., 2002). The degree of RO concentration depends on prevailing energy costs: if it is to be used as a pre-concentrator, it is probably best to do a 2-folds concentration by RO to 12 % total solids (TS), and the rest (12-45 % TS) by evaporation. The limiting factors in RO are osmotic pressure, viscosity and solubility of the lactose and calcium salts present in whey, which may precipitate out especially if temperature and pH are not regulated (Cheryan and Alvarez, 1995).

There are currently three ways to obtain demineralization of whey: electrodialysis, ion-exchange and nanofiltration. Electrodialysis (ED) could be an attractive alternative for demineralization of milk products and whey to be used in infant formulas and special dietary products instead of NF. Since the demineralization rate and efficiency, as well as costs, depend on the conductivity of the solutions, whey is usually concentrated prior to electrodialysis. This also reduces the processing volume. However, electrodialysis cannot complete a 100 % demineralization because of the rapid increase in resistance as the ions are being depleted. It appears that the best option is to use ED to obtain 50-70 % demineralization, followed by ion-exchange up to the required amount (Cheryan and Alvarez, 1995). Due to the high salt content of whey, ion-exchange is operated for short time, with frequent regeneration of the resin, which involves large amounts of regeneration chemicals and water to rinse excess chemicals from the weak anion resin. Substances with molar masses higher than 300 g mol⁻¹ and multivalent ions are (partially) retained by NF membranes. With NF, small molecules, such as NaCl, are removed along with water whereas other material, such as lactose, proteins and fat are retained, making it suitable for desalting whey instead of electrodialysis or ion-exchange (Rosenberg, 1995).

Manufacture and use of functional whey components

Membrane processes have been successfully implemented at large commercial scale in the dairy industry (Rosenberg, 1995; Zydney, 1998). The WPC recovered by UF have created a whole new commodity market where they are used as ingredients in a variety of formulated products, such as dairy, bakery, meat, beverage and infant formula products, due to the excellent functional properties of their proteins. Moreover, WPC production represents the best means for the utilization of whey proteins (Madureira et al., 2007; Smithers, 2008; Chicón et al., 2009). From a functional and nutritional point of view, both MF and ion-exchange are able to result in high-purity WPI. At equivalent protein contents, microfiltered WPI tends to be lower in fat content, while ion-exchange WPI tends to be lower in lactose, which designates the "nutritional" or "nutraceutical" value (Li et al., 2005).

Most of the studies have focused on the preparation of enriched α -La and/or β -Lg fractions, since these two proteins account for more than two-thirds of the total whey proteins (Amundson et al., 1982; Cheang and Zydney, 2003). β-Lg, isolated by combining membrane separation with ion-exchange, shows excellent gel formation and foaming properties, and can be used as a texturizing and stabilizing agent because of its capability in structuring dairy product matrices such as yoghurt or cream cheese (Saufi and Fee, 2009). Purified α -La by a two-step process of UF followed by precipitation, results in a decreased content of contaminants (bovine serum albumin, immunoglobulins) down to zero and enhanced α -La/ β -Lg ratio, and can be used to replace ingredients, such as soy protein, egg whites, or gelling agents, to provide high gel strength, viscosity, aeration, water binding, and high solubility in infant formulas and meat products (Jost et al., 1999; Muller et al., 2003). Isolation of IgGs from whey using UF and immobilized metal affinity chromatography showed higher recovery and purity than UF with ion-exchange chromatography (Fukumoto et al., 1994). As having multiple biological properties including antimicrobial, anti-inflammatory, anticarcinogenic, immuno-modulatory, and bone growth factor properties (Wong et al., 1997; Uchida et al., 2005) lactoferrin isolation and purification is another challenge in implementation of membrane separations in whey utilization. LF extraction from skim milk or whey by cation-exchange chromatography LF isolates of high purity (>90 % protein), which are nowadays commercially available. However, this process has some limitations at industrial scale due to high cost and relatively low throughputs. Among different strategies that have been investigated, electrically-enhanced UF or cross-flow MF could represent an interesting alternative to chromatography (Brisson et al., 2007; Lu et al., 2007).

Conclusion

Membrane technology has been commercially integrated in dairy industry for more than fourty years, and the fascinating new applications are expected in the next decade. The cheese industry was one of the first to explore the possibilities of using membrane-concentrated milk for cheese quality improvement. Technological advances related to the development of new membranes, improvements in process engineering and better understanding of the functionality of milk constituents have extended the range of membrane separation applications in dairy industry. Provided some solid evidence of functionality of individual whey components a sustainable market can be expected, however, keeping in mind that using membrane separation often means compromising between selectivity and productivity. Therefore, challenging developments in membrane processes should focus on efficient fractionation of minor whey components of desired biological and nutraceutical properties.

Membranski procesi u proizvodnji funkcionalnih sastojaka sirutke

Sažetak

Posljednjih godina sirutka je prepoznata kao značajan izvor nutritivnih i funkcionalnih sastojaka u prehrambenoj industriji. Komercijalni sirutkini proizvodi uključuju različite tipove sirutke u prahu, koncentrate proteina sirutke i izolate, te frakcionirane proteine, kao što su α -laktalbumin and β -laktoglobulin. Povećani interes u separaciji i frakcinaciji proteina sirutke dolazi iz različitosti njihovih funkcionalnih, bioloških i nutritivnih svojstava. Zbog zabrinutosti radi ekoloških aspekata, istraživači su se fokusirali na tehnologiju membranske separacije, što osigurava nove mogućnosti za široki raspon separacije proteina i laktoze. Membranska separacija je proces u kojem se čestice odvajaju prema molekularnoj veličini. Tipovi membranskih procesa su ultrafiltracija, mikrofiltracija, reverzna osmoza, elektrodijaliza i nanofiltracija. Veća pročišćenost sirutkinih proteina moguća je kombiniranjem membranske separacije s ionskom izmjenom. Ovaj rad prikazuje pregled mogućih kombinacija membranske separacije u preradi sirutke i proizvodnji funkcionalnih sastojaka koji potječu iz sirutke.

Ključne riječi: sirutka, membranski procesi, funkcionalni spojevi

References

- Amundson, C.H., Watanawanichakorn, S., Hill, C.G. (1982): Production of enriched protein fractions of β-lactoglobulin and α-lactalbumin from cheese whey. *Journal of Food Processing and Preservation* 6, 55-71.
- Atkinson, S. (2003): Membrane filtration aids cheesemaking process. *Membrane Technology* 12, 9.
- Atra, R., Vatai, R.G., Bekassy-Molnar, E., Balint, A. (2005): Investigation of Ultra- and Nanofiltration for utilization of whey protein and lactose. *Journal of Food Engineering* 67, 325-332.
- Baker, R.W., Wijmans, J.G., Athayde, A.L., Daniels, R., Ly, J.H., Le, M. (1997): The effect of concentration polarization on the separation of volatile organic compounds from water by pervaporation. *Journal of Membrane Science* 137, 159-172.
- Balannec, B., Gösan-Guiziou, G., Chaufer, B., Rabiller-Baudry, M., Daufm, G. (2002): Treatment of dairy process waters by membrane operations for water reuse and milk constituents concentration. *Desalination* 147, 89-94.
- Baruah, G.L., Venkiteshwaran, A., Belfort, G. (2005): Model for optimizing crossflow microfiltration and ultrafiltration processes: A New Predictive and Design Tool. *Biotechnol Prog 21*, 1013.
- Bhushan, S., Etzel, M.R. (2009): Charged ultrafiltration membranes increase the selectivity of whey protein separations. *Journal of Food Science* 74 (3), 131-139.
- Brans, G., Schro

 en, C.G.P.H., Van der Sman, R.G.M., Boom, R.M. (2004): Membrane fractionation of milk: state of the art and challenges. *Journal of Membrane Science* 243, 263-272.
- Brisson, G., Britten, M., Pouliot, Y. (2007): Electricallyenhanced crossflow microfiltration for separation of lactoferrin from whey protein mixtures. *Journal of Membrane Science* 297 (1-2), 206-216.
- Cheang, B., Zydney, A.L. (2003): Separation of α-lactalbumin and β-lactoglobulin using membrane ultrafiltration. *Biotechnology and Bioengineering* 83, 201-209.

- Cheryan, M. (1998): Ultrafiltration and Microfiltration Handbook. Technomic Publishing Company, Lancaster, PA 527 p.
- Cheryan, M., Alvarez, J.R. (1995): Food and beverage industry applications. In *Membrane Separations Technology*. Noble, R.D., Stern, S.A., Eds, Elsevier Science, NY, 415-465.
- Chicón, R., Belloque, J., Alonso, E., López-Fandino, R. (2009): Antibody binding and functional properties of whey protein hydrolysates obtained under high pressure. *Food Hydrocolloids* 23 (3), 593-599.
- Chiu, C.K., Etzel, M.R. (1997): Fractionation of lactoperoxidase and lactoferrin from bovine whey using a cation exchange membrane. *Journal of Food Science* 62, 996-1000.
- Cuartas-Uribe, B., Alcaina-Miranda, M.I., Soriano-Costa, E., Mendoza-Roca, J.A., Iborra-Clar, M.I., Lora-García, J. (2009): A study of the separation of lactose from whey ultrafiltration permeate using nanofiltration. *Desalination 241* (1-3), 244-255.
- DaCosta, A.R., Fane, A.G., Wiley, D.E. (1993): Ultrafiltration of whey protein solutions in spacer-filled flat channels. *Journal of Membrane Science* 76, 245-254.
- Del Re, G., DiGiacomo, G., Aloisio, L., Terreri, M. (1998): RO Treatment of waste waters from the dairy industry. *Desalination* 119, 205-206.
- Doultani, S., Turhan, K.N., Etzel, M.R. (2004). Fractionation of proteins from whey using cation exchange chromatography. *Process Biochemistry* 39, 1737-1743.
- Fukumoto, L.R., Li-Chan, E., Kwan, L., Nakai, S. (1994): Isolation of immunoglobulins from cheese whey using ultrafiltration and immobilized metal affinity chromatography. *Food Research International* 27, 335-348.
- 20. Gerberdingand, S.J., Byers, C.H. (1998). Preparative ion-exchange chromatography of proteins from dairy whey. *Journal of Chromatography A* 808, 141-151.
- Greiter, M., Novalin, S., Wendland, M., Kulbe, K.D., Fischer, J. (2002): Desalination of whey by electrodialysis and ion-exchange resins: analysis of both processes with regard to sustainability by calculating their cumulative energy demand. *Journal of Membrane Science 210*, 91-102.
- Ha, E., Zemel, M.B. (2003): Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people. *Journal of Nutritional Biochemistry* 14, 251-258.
- Jost, R., Maire, J.C., Maynard, F., Secretin, M.C. (1999): Aspects of whey protein usage in infant nutrition: A Brief Review. *International Journal of Food Science and Technology* 34, 533-542.
- 24. Kinsella, J.E., Whitehead, D.M. (1989): Proteins in whey: Chemical, physical, and functional properties. *Advances in Food and Nutrition Research* 33, 343-438.
- Kosikowski, F.V., Mistry, V.V. (1997): Cheese and Fermented Milk Foods. Volume 1. Origins and Principles. 3rd ed. Kosikowski, F.V. Ed., Westport, Connecticut. 422-453, 500-519.

- Li, C.P., Enomoto, H., Ohki, S., Ohtomo, H., Aoki, T. (2005): Improvement of functional properties of whey protein isolate through glycation and phosphorylation by dry heating. *Journal of Dairy Science* 88, 4137-4145.
- Lu, R.R., Xu, S.Y., Wang, Z., Yang, R.J. (2007): Isolation of lactoferrin from bovine colostrum by ultrafiltration coupled with strong cation exchange chromatography on a production scale. *Journal of Membrane Science* 297 (1-2), 152-161.
- McClements, D.J., Decker, E.A., Park, Y., Weiss, J. (2009): Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. *Critical Reviews in Food Science and Nutrition* 49 (6), 577-606.
- Madureira, A.R., Pereira, C.I., Gomes, A.M.P., Pintado, M.E., Malcata, F.X. (2007): Bovine whey proteins -Overview on their main biological properties. *Food Research International* 40 (10), 1197-1211.
- Maubois, J.L. (1997): Current uses and future perspectives of MF technology in the dairy industry. *International Dairy Federation Bulletin* 320, 37-40.
- Muller, A., Chaufer, B., Merin, U., Daufin, G. (2003): Purification of α-Lactalbumin from a prepurified acid whey: Ultrafiltration or Precipitation. *Lait* 83, 439-451.
- Nelson, B.K., Barbano, D.M. (2005): A microfiltration process to maximize removal of serum proteins from skin milk before cheese making. *Journal of Dairy Sci*ence 88, 1891-1900.
- Pacheco, M.T.B., Amaya-Farfan, J., Sgarbieri, V.C. (2002): Partial characterization of a whey protein concentrate and its enzyme hydrolysates. *Journal of Food Biochemistry* 26, 327-339.
- Pouliot, Y. (2008): Membrane processes in dairy technology from a simple idea to worldwide pacea. *International Dairy Journal 18*, 735-740.
- Rektor, A., Vatai, G. (2004): Membrane filtration of mozzarella whey. *Desalination* 162, 279-286
- Rosenberg, M. (1995): Current and future applications for membrane processes in the dairy industry. *Trends in Food Science and Technology* 6, 12-19.
- Saboya, L.V., Maubois, J.L. (2000): Current developments of microfiltration technology in the dairy industry. *Lait* 80 (6), 541-553
- Saufi, S.M., Fee, C.J. (2009): Fractionation of β-Lactoglobulin from whey by mixed matrix membrane ion exchange chromatography. *Biotechnology and Bioen*gineering 103 (1), 138-147.
- Saxena, A., Tripathi, B.P., Kumar, M., Shahi, V.K. (2009): Membrane-based techniques for the separation and purification of proteins: An overview. *Advances in Colloid and Interface Science* 145 (1-2), 1-22.
- Smith, K.E. (2001): Background on milk protein products. In *Dairy Proteins*. Bishop, R., Mathison, M., Smith, K.E. Eds. Wisconsin Center for Dairy Research, 5-8.
- 41. Smithers, G.W. (2008): Whey and whey proteins -From "gutter-to-gold". *International Dairy Journal 18* (7), 695-704.

- 42. Tolkach, A., Kulozik, U. (2005): Fractionation of whey proteins and caseinomacropeptide by means of enzymatic crosslinking and membrane separation techniques. *Journal of Food Engineering* 67, 13-20.
- Uchida, T., Oda, T., Sato, K., Kawakami, H. (2005): Availability of lactoferrin as a natural solubilizer of iron for food products. *International Dairy Journal* 16, 95-101.
- 44. Van Reis, R., Zydney, A. (2007): Bioprocess membrane technology. *Journal of Membrane Science* 297 (1-2), 16-50.
- 45. Van der Horst, H.C., Hanemaaijer, J.H. (1990): Crossflow microfiltration in the food industry. State of the Art. *Desalination* 77, 235-258.
- Wong, C.W., Seow, H.F., Husband, A.J., Regester, G.O., Watson, D.L. (1997): Effects of purified bovine whey factors on cellular immune functions in ruminants. *Veterinary Immunology and Immunopathology* 56, 85-96.

- Yorgun, M.S., Akmehmet Balcioglu, I., Saygin, O. (2008): Performance comparison of ultrafiltration, nanofiltration and reverse osmosis on whey treatment. *Desalination 229* (1-3), 204-216.
- Zadow, J.G. (1994): Utilisation of milk components: Whey. In *Modern Dairy Technology*. Robinson, R.K., Ed., 2nd edition. Chapman and Hall, London. 313-374.
- 49. Zeman, L.J., Zydney, A.L. (1996): Microfiltration and Ultrafiltration: Principles and Applications. Marcel Dekker Inc., New York, 618 p.
- Zydney, A.L. (1998): Protein separations using membrane filtration: New opportunities for whey fractionation. *International Dairy Journal* 8, 243-250.