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Research Paper

Optimization of Cheese Whey Ultrafiltration/Diafiltration for the Production of Beverage Liquid Protein Concentrates with Lactose Partially Removed

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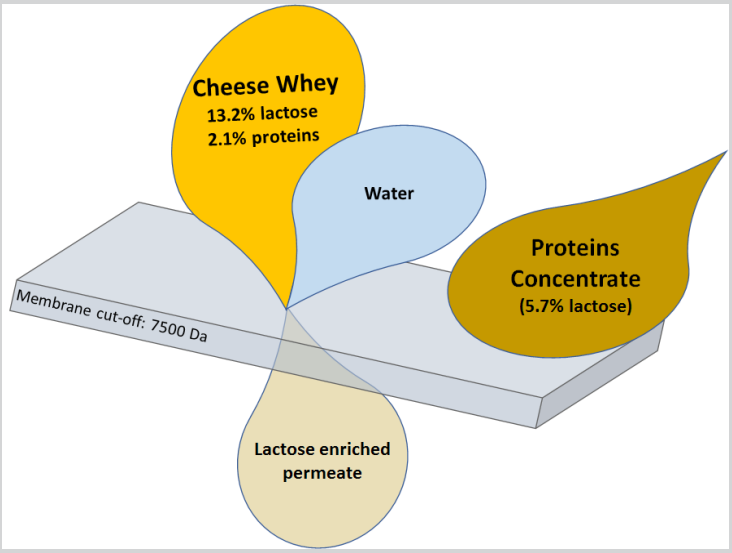
Highlights

- Cheese whey protein concentration & lactose removal by ultrafiltration/ diafiltration
- Optimization of diafiltration: water volumes and frequency of addition
- Production of whey protein concentrates (WPCs)
- Ultrafiltration (UF)/diafiltration (DF) for the production of WPCs for Beverages
- UF/DF for high yield production of traditional portuguese “Requeijão”

Graphical abstract



Abstract

The processing of cheese whey pre-concentrated by reverse osmosis has been carried out through ultrafiltration in diafiltration mode to produce whey protein concentrates with lower lactose content to be incorporated in beverages. The initial cheese whey protein and lactose contents were 2.13g/100g and 13.22g/100g, respectively. The commercial membranes, GR95PP, supplied by Alfa Laval, Denmark, were characterized in terms of a hydraulic permeability of 1.21 L/(h·m²·bar) and a molecular weight cut-off of 7500 Dalton. The permeation tests were carried out in a plate and frame Lab-Unit 20 from Alfa Laval, Denmark, and a membrane surface area of 0.072 m² was installed. The ultrafiltration of cheese whey in total recirculation mode yielded two asymptotic variations of the permeate fluxes versus the transmembrane pressure. For operating pressures up to 12 bar the permeate flux increases linearly with the pressure. Then, with the increasing pressure, they deviate from linearity and reach a limiting flux of 8.79 L/(h·m²·bar) at 30 bar. The slope of the asymptotic linear variation is 0.48 L/(h·m²·bar). To have minimal effects of concentration polarization the operating pressure was set-up at 12 bar. The optimization of ultrafiltration/diafiltration was carried out in terms of the volumetric concentration factors and the frequency of diavolumes addition. At a volumetric concentration factor of 1.32 the lactose content decreased from 13.22% to 5.7%.

1. Introduction

Dairy industry has been pioneer in the use of membrane processes, namely pressure driven membrane processes, to concentrate, clarify and fractionate their products. The four main processes of microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) have contributed to the simplification of traditional processes and to make the products or the processes more economically competitive and environmentally friendly [1].

The membrane technology enables the removal of unwanted compounds, such as microorganisms and drugs, giving a more interesting texture to the final product, as well as an increase in shelf life time. It also allows the

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processing of dairy products without protein denaturation and minimizes changes in the sensorial attributes of the products [2]. An example is the application of MF in the reduction of the number of bacteria and spores, which is called *cold pasteurization*. This alternative does not affect the taste of the milk and can provide a longer shelf life than the one offered by milk pasteurization [3].

From the sixties onwards, the increase in cheese consumption has attracted the special attention of producers to membrane technology [4]. On the other hand the cheese whey, drained from the manufacture of cheese, is considered a by-product of high biological and economical value due to its high content in essential amino acids, with functional properties and with application in food and pharmaceutical industries [5,6]. However, the cheese whey is also a serious polluting waste due to its high organic load [5]. For this reason, its reuse is now an industrial practice with economic and environmental benefits and made the concentration of the whey by RO and/or by UF, one of the first commercial applications of membrane technology [3]. Nowadays, it is estimated that about 2/3 of membrane technology, that serves this industry, is used for the treatment of whey and about 1/3 has application in milk processing [2].

According to statistical data, the worldwide production of whey is about 180 to 190×10^6 ton/year [5]. It is estimated that 40-50% of this value is drained into the sewage and therefore causing severe environmental problems. The remaining percentage is processed as animal feed or human food [5], of which half is used in liquid form, 30% in form of whey powder, 15% in the manufacture of lactose and the rest as whey protein concentrate [7]. In fact, the production of about 1-2 kg of cheese yields 8-9 kg of whey [5], representing 85-90% of milk volume used in cheese production and containing about 55% of milk nutrients, such as soluble globular proteins, lactose, mineral salts and vitamins [8].

Whey protein concentrates (WPCs) are the most important commercial whey products, being up to 40 times greater than whey powder [7]. They may contain 35% to 85% protein in the total solids content and this can be achieved by a combination of UF and diafiltration (DF). The whey protein isolates (WPIs), which may contain 90% of protein in relation to the total solids content, use the MF process for the removal of fat and bacteria [9,10].

The application of membrane processes to the manufacture of WPCs is an alternative to the conventional method of thermal evaporation. They have the advantage of not using heat, which makes the processes more economical while avoiding the denaturation of the whey proteins [2].

In addition, the whey ultrafiltration process produces a permeate rich in nutrients, which represents an opportunity for its valorisation: its content in lactose can be applied in the sweet or pharmaceutical industries, it has nutrients that are important for fermentation medium allowing the manufacture of high value biological products and its content in oligosaccharides have potential application in human nutrition [6].

The versatility of ultrafiltration carried out in diafiltration mode allows the production of WPCs with different degrees of purity, namely in terms of lactose and salts content, to be used in the beverage-processing industry as functional proteins or protein hydrolysates to adjust the nutritional value, cost, physical and sensory properties and shelf life increase [10,11].

The functional properties of WPCs such as viscosity enhancement, foaming ability, emulsifying effects, water absorption and gel formation, are beneficial in the production of beverages. Whey proteins also possess important nutritional and biological properties allowing the promising production of functional beverages [12,13], with therapeutical and medicinal properties associated with these proteins [12,14–16]. Currently, whey-based fruit beverages are the most commonly consumed beverages. They are able to be prepared from a vast range of fruits [17,18].

The production of "Requeijão" (a Portuguese traditional whey cheese) from WPCs represents a strong asset in terms of higher manufacturing yields and energy minimization. In fact, the RO whey concentration is used in a Portuguese cheese factory to reduce waste whey volume with some water re-use. The "Requeijão" production is based on whey protein heat coagulation (80-90 ºC) which is a high energy consuming process. Whey concentration seems to be a good way to reduce the process overall energy consumption, promoting lower whey volume and increasing process yield. The remaining problem with RO whey concentrate for "Requeijão" production is claimed to be the textural properties which can be due to the concentrate high lactose content. In fact, different sugars inhibit unfractionated whey proteins, and the more effective are disaccharides, lactose and sucrose [19]. According to the authors, sucrose promotes the whey protein denaturation but inhibits their subsequent aggregation increasingly with the increase in sugar concentration. This is an important issue of milk stability in heat treatment but should be a negative issue in "Requeijão" production as protein aggregation is an essential step.

The present work aims the development of an hybrid process of Ultrafiltration /Diafiltration to optimize the composition of WPCs namely in terms of the simultaneous reduction of lactose content and increase of the protein content to comply with the requisites for incorporation in beverages and for the production of "Requeijão" .

2. Experimental

2.1. Cheese Whey

The cheese whey to be processed was previously concentrated by reverse osmosis in a Portuguese dairy industry, Queijo Saloio S.A., located in Ponte de Rol, Torres Vedras; Portugal. The feed of the RO unit is a cheese whey that comes from cheese production using different goat, cow and sheep milk mixes and therefore the RO whey concentrates reflect a very complex composition as shown in Table 1. An aqueous solution of hydrogen peroxide was added to the whey in order to preserve it [20] until cold refrigeration. Subsequently, it was stored in a refrigeration chamber with the temperature kept at 4 °C.

2.2. Membranes and filtration unit

The commercial ultrafiltration membranes, GR95PP, were supplied by Alfa Laval, Denmark. A schematic illustration of the permeation unit is displayed in Figure 1.

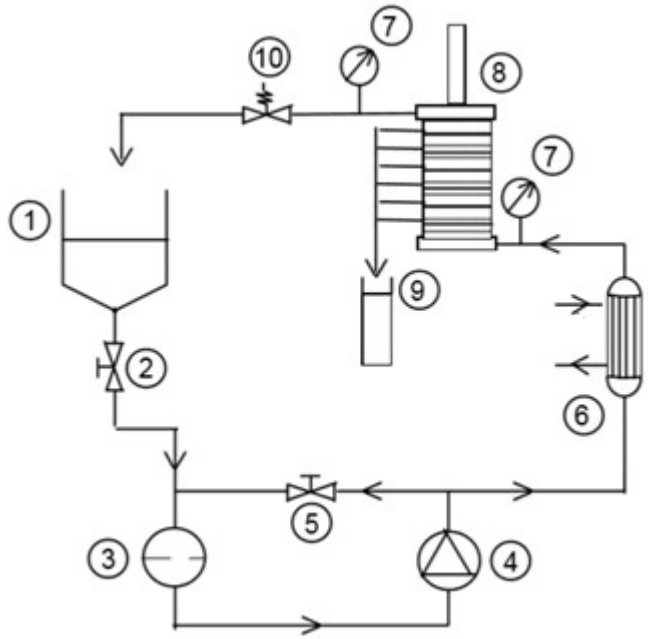

Fig. 1. Plate and frame Lab-Unit 20 from Alfa Laval, Denmark: 1) Feed reservoir; 2) Valve; 3) Filter; 4) Cross-flow pump; 5) Valve; 6) Heat exchanger; 7) Manometer; 8) Membrane module; 9) Collecting permeate; 10) Pressure control valve.

2.3. Experimental procedure

A membrane area of 0.072 m^2 is installed in the Lab Unit 20 (see Figure 1). To eliminate fluctuations and assure reproducibility in the essays, permeation of pure water is carried out at a pressure that is approximately 20% above the highest operating pressure to be used and for a period of 3 hours.

The permeation properties of the membranes were assessed through the hydraulic permeability, L_p, the apparent rejection coefficients to a set of reference solutes and the molecular weight cut-off, MWCO. The L_p was measured with a transmembrane pressure ranging from 1.5 to 8 bar, at 25 ºC, considering maximum circulation flow. The apparent rejection coefficient, f, is defined as $f=(C_b-C_p)/(C_b)$, where C_b and C_p are the feed and the permeate concentrations, respectively. The apparent rejection coefficients were determined with the recirculation of the retentate and the permeate streams, at a maximum circulation flow, for solutions of 500 mg/L of a monovalent salt,

NaCl, bivalent salts, $CaCl_2$ and Na_2SO_4, and an organic solute, lactose. The feed is pressurized at 4 bar and is kept at the temperature of 25 °C. The MWCO calculation is based on the results of permeation experiments of solutions of 500 mg/L of reference solutes (1, 4 and 6 kDa polyethylene glycols). The feed is pressurized at 4 bar, circulates at the maximum circulation flow and is kept at the temperature of 25 °C.

A cheese whey permeation run with retentate and permeate streams recirculated to the feed reservoir and with the transmembrane pressure varying from 1 to 40 bar is carried out with the purpose of selecting the operating pressure in the region of the linear variation of the permeate fluxes with the transmembrane pressure.

The operating pressure of 12 bar was selected for the permeation experiments of cheese whey concentration. The volumetric concentration factor, VCF, is defined as the ratio of the feed volume in the feed tank at the beginning (V_i) and at the end (V_f) of the concentration operation, $VCF=V_i/V_f$. The VCF was varied from 1 up to 2.

The ultrafiltration (UF)/diafiltration (DF) permeation experiments were also performed at 12 bar. The VCF is now defined as the feed initial volume plus the DF added volumes divided by the feed final volume.

Prior to sample collection, the circulation streams are stabilized for 30 minutes.

Membrane cleaning followed the permeation experiments. This was performed with deionized water at 40 ºC until a 90% permeate flux recovery. In the case of that being not reached a 0.1% Ultrasil 10 solution is circulated for 15 minutes at 40 ºC.

2.4. Physicochemical characterization of the cheese whey

The cheese whey sample was object of the following determinations: Acidity (AOAC official method 947.05, [21]); pH (Metrohm pH meter); lactose (Lactose = Total solids - Ash-Protein - Fat content (1)); total solids (AOAC official method 925.23A, [21]), total nitrogen by the Kjeldahl method, being the protein calculated from the determination of total nitrogen [IDF 020-3/ISO 8968-2 (2001)]; ash (AOAC official method 945.46, [21]), fat [determined by the Gerber method, according to IDF 226/ISO 2446 (2008)]; chloride by Charpentier-Volhard method, according to the Portuguese standard method NP – 471 (1983), the content of other mineral elements, such as phosphorus, sodium, potassium, calcium and magnesium were determined from the ash residue, after treatment with HCl (6N), with heat and subsequent filtering; the mineral elements were determined in solution, in the Laboratório Químico Agrícola Rebelo da Silva (INIAV), by ICP-OES (Thermo, Unicam, Mod.ÍRIS Intrepid II XSP Radial).

3. Results and discussion

3.1. Physicochemical composition of cheese whey

Table 1 shows the physicochemical composition of the RO concentrated cheese whey that would be subjected to UF/DF processing.

Table 1
Cheese whey characterization.

Parameter	Value
pH	5.71
Acidity (g lactic acid/L)	4.92
Protein (g/100g)	2.13
Ash (g/100g)	1.35
Chloride (g NaCl/100g)	0.59
Fat (g/100g)	0.30
Total solids (g/100g)	17.00
Lactose (g/100g)	13.22
Calcium (mg/100g)	97.67
Phosphate (mg/100g)	97.33
Sodium (mg/100g)	116.67
Potassium (mg/100g)	501.00
Magnesium (mg/100g)	21.33

The high values of total solids, protein and lactose (17.00 g/100 g, 2.13 g/100 g and 13.22 g/100 g, respectively) are due to the fact that this cheese whey has been pre-treated by reverse osmosis (RO). The literature average values for total solids, protein and lactose are 6.25 g/100 g, 0.67 g/100 g and 4.39 g/100g, respectively. One should note, also, that the fat content, 0.30 g/100 g, is lower than the typical value of 0.75 g/100g for non-processed cheese whey as the present one was subjected to a pre-treatment for fat removal.

3.2. Membrane characterization

3.2.1. Hydraulic permeability

Figure 2 represents the variation of pure water flux, J_w, versus the transmembrane pressure, ΔP. The slope of this linear variation, $J_w=1.21.\Delta P$, is the membrane hydraulic permeability of 1.21 $L/(h.m^2.bar)$.

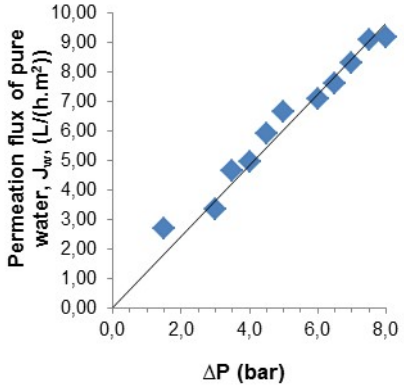

Fig. 2. Permeate flux of pure water vs. the transmembrane pressure. GR95PP membrane. Installation Lab-Unit 20. Membrane surface area: 0,072 m^2; Temperature: 25 ºC; Maximum circulation flow.

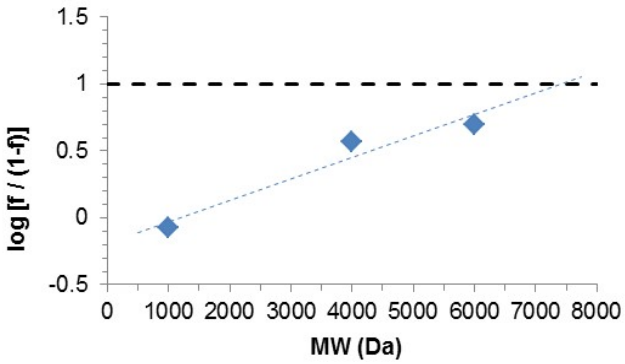

Fig. 3. Apparent rejection coefficients of reference solutes vs. solute molecular weight. Installation Lab-Unit 20. Membrane surface area: 0,072 m^2; Temperature: 25 ºC; Maximum circulation flow.

3.2.2. Determination of the molecular weight cut-off (MWCO)

Figure 3 represents the determination of the MWCO = 7500 Da by the intersection of the curve of $log(f/(1-f))$ *vs* solute molecular weight and the 91% rejection line ($log(f/(1-f)) = 1$).

3.2.3. Apparent Rejection Coefficients to Salts and Lactose

The Table 2 displays the apparent rejection coefficients to a monovalent salt, NaCl, bivalent salts, $CaCl_2$ and Na_2SO_4, and lactose.

3.3. Variation of ultrafiltration permeate flux with transmembrane pressure in total recirculation mode

Figure 4 shows that the permeate flux increases linearly with the pressure, in the range from 1 to 12 bar, and then deviates from linearity and reaches a limiting flux of 8.79 L/(h.m^2) at 30 bar. The slope of the asymptotic linear variation is 0.48 L/(h.m^2.bar) and it compares with the higher value of 1.21 L/(h.m^2.bar) of the hydraulic permeability. In order to have minimal effects of concentration polarization the operating pressure in the subsequent experiments in concentration mode is set to 12 bar.

Table 2
Apparent rejection coefficients to salts and lactose.

Solute	*f* (%)
NaCl	13
$CaCl_2$	47
Na_2SO_4	70
Lactose	18

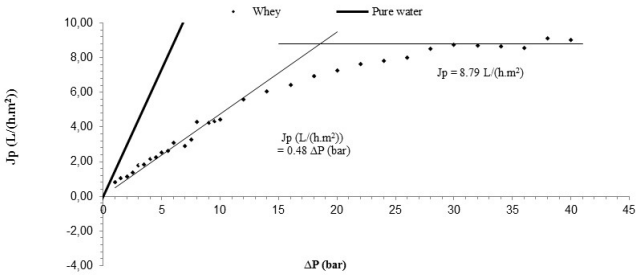

Fig. 4. Variation of cheese whey permeation flux (Jp) with transmembrane pressure (ΔP). Installation Lab-Unit 20. Membrane GR95PP; Membrane surface area: 0.072 m^2; Temperature: 25ºC; Feed circulation velocity: 0.94m/s.

3.4. Variation of ultrafiltration permeate flux with volume concentration factor (VCF) in concentration mode

Figure 5 displays, in concentration mode, the variation of the permeation flux *versus* the VCF. The initial feed volume is 5 L. The decrease of the permeate flux with the increase of VCF follows two asymptotic behaviours: a linear and steep decrease for low values of VCF and a plateau which is reached at VCF values higher than 1.5 and up to 2. This plateau has the value of 2.34 L/h.m^2. For a VCF of 2, the reduction of the permeation fluxes is 31%. The protein and lactose apparent rejection coefficients, for the volumetric concentration factor of 2, were 89% and 55%, respectively. These values indicate almost complete retention of protein and an increase in the retention coefficient to lactose from 18% (see Table 2) to 55%. To promote the passage of lactose to the permeate stream the ultrafiltration will be operated in diafiltration mode.

3.5. Ultrafiltration in diafiltration mode

In order to decide about a diafiltration strategy in terms of diavolumes and their adittion frequency a preliminary test is carried out to evaluate the permeate flux decline, the lactose passage to the permeate and protein retention.

3.5.1. Preliminary assessment of diafiltration

Figure 6 shows an initial flux decline of 15%. Taking this into account the diavolumes were added at 16% and 4% of the permeate flux decline (VCF of 1.05 and 1.25, respectively). The water volumes introduced were 20% of the feed tank volume at the time of addition. Figure 6 represents the evolution with time of the permeation flux and the feed volume. Despite a slight increase of the permeate flux at the first water addition there was not a significant improvement due to the strong flux decline at the very beginning of the ultrafiltration operation, J_p=-3.30.t+5.20 ($0 \leq t \leq 0.236$ h). Moreover, the variation with VCF of the apparent rejection coefficients to lactose is shown in Table 3.

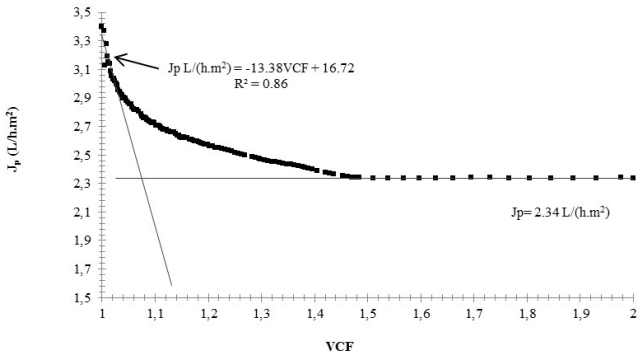

Fig. 5. Variation of cheese whey permeation fluxes with VCF, until VCF=2. Installation Lab-Unit 20. Membrane GR95PP. Membrane surface area: 0.072 m^2; Transmembrane pressure: 12 bar; Temperature: 25ºC; Feed circulation velocity: 0.94m/s.

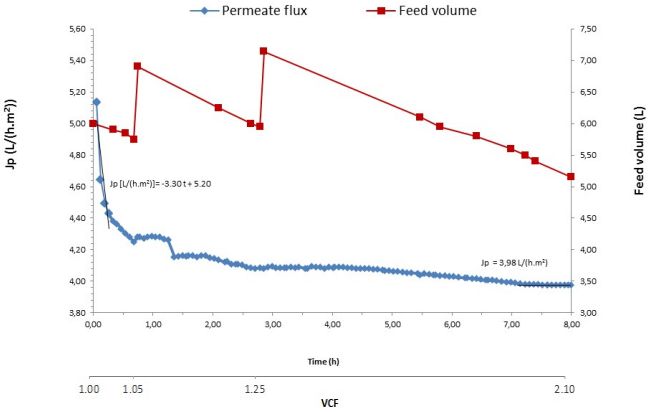

Fig. 6. Variation of permeation flux (Jp) and feed volume with time. Installation Lab-Unit 20. Membrane GR95PP; Membrane surface area: 0.072 m^2; Transmembrane pressure: 12 bar; Temperature: 25 ºC; Feed circulation velocity: 0.94m/s.

Table 3
Variation of the apparent rejection coefficients to lactose and protein.

VCF	Lactose (%)	Protein (%)
1.05	33	74
1.25	29	89
2.10	16	80

The results indicate that the rejection to lactose tends to decrease with the increase of the volumetric concentration factor. It can be concluded that the diafiltration improved lactose permeation, without compromising the protein concentration.

In order to improve the permeate flux, a pre-dilution of the cheese whey feed was implemented in the subsequent UF/DF optimization.

3.5.2. Optimization of UF/DF

3.5.2.1. Influence of initial dilution and of diavolumes

Figure 7 shows the permeate fluxes evolution with time considering that at first a pre-dilution was carried out through the addition of 2 L of water to the 5 L of the cheese whey charged to the feed tank. Secondly, in the course of operation, at 16% flux decline or *VCF* = 1.25, water was added in the same percentage of 40%.

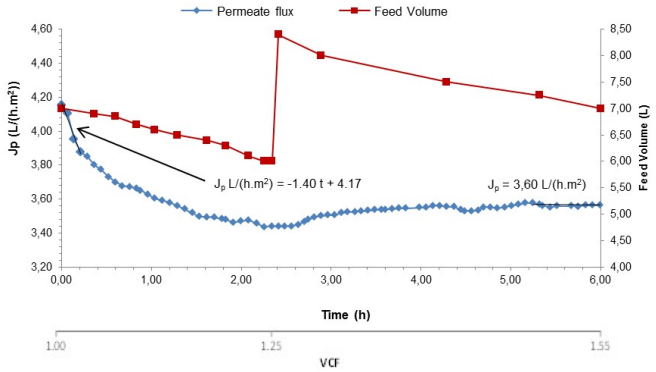

Fig. 7. Variation of the permeation flux (J_p) and the feed volume with time. Installation Lab-Unit M20. Membrane GR95PP; Membrane surface area: 0.072 m^2; Transmembrane pressure: 12 bar; Temperature: 25 ºC; Feed circulation velocity: 0.94m/s.

Figure 7 shows that the initial permeation flux has a linear asymptotic behaviour until 0.233 h, $J_p = -1.40.t + 4.17$. In the preliminary test of UF/DF, the permeation flux decline is given by $J_p = -3.30.t + 5.20$, with a steeper slope. The variation with VCF of the apparent rejection coefficients to lactose and protein is shown in Table 4.

One should note that although the removal of lactose is improved, the protein rejection coefficient at $VCF = 1.25$ is 56%, a lower value when compared to 89% in the preliminary test (see Table 3).

3.5.2.2. Influence of diavolumes addition frequency

Before starting the operation there was a cheese whey dilution by the addition of 20% of pure water relative to the volume present in the feed tank. During the operation, 20% of pure water relative to the volume observed in the feed tank was added at 10, 20, 30 and 40 minutes (see Figure 8). More frequent successive additions of water not only interrupt the decrease of permeation fluxes but also promote their increase. The variation of cheese whey permeation flux over time shows a linear asymptotic behaviour until 0.210 h, $J_p = -1.07.t + 4.39$. It is concluded that the initial decrease of permeation flux in this test is significantly lower than the decrease observed in the UF/DF preliminary evaluation test.

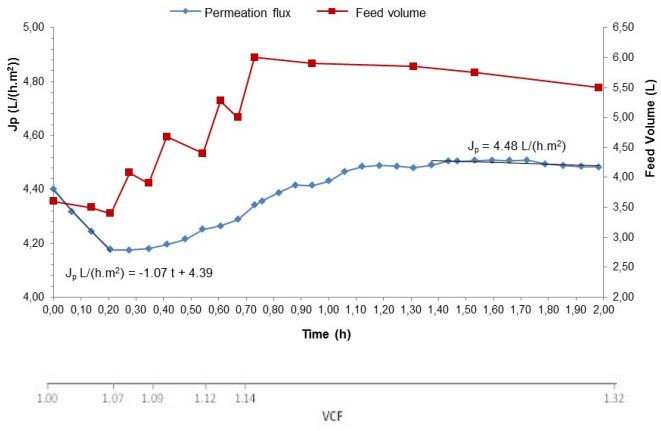

Fig. 8. Variation of the permeation flux (Jp) and the feed volume with time. Installation Lab-Unit 20. Membrane GR95PP. Membrane surface area: 0.072 m^2; Transmembrane pressure: 12 bar; Temperature: 25 ºC; Feed circulation velocity: 0.94m/s.

The variation of the apparent rejection coefficients to lactose and protein upon the VCF increase is shown in Table 5. It is verified that the rejection coefficient to the protein increases whereas the one to lactose decreases with the increase of VCF. Successive additions of pure water, in short time intervals, led to high protein rejection coefficients, closer to total retentions.

The lactose content in the protein concentrates decreased as diafiltration proceeds and the VCF increases, that is: from the initial lactose content of 13.2% (see Table 1) a value of 5.7% is reached at a VCF of 1.32. This is a common lactose concentration in cheese whey used for "Requeijão" production.

4. Conclusions

Throughout this work, a process of ultrafiltration in diafiltration mode was developed and optimized to obtain protein concentration and lactose removal. It was found that the addition of pure water increased the permeation fluxes and enhanced the passage of solutes, which, according to the membrane MWCO, should not be retained, like lactose. The reduction of lactose content in whey protein concentrates is an important issue when their use is envisaged to the preparation of whey based beverages, given the widespread detection of lactose intolerance, as well as for "Requeijão" production, given the inhibition effect of lactose on protein heat coagulation.

Table 4
Variation of the apparent rejection coefficients to lactose and protein.

VCF	Lactose (%)	Protein (%)
1.25	18	56
1.55	11	73

Table 5
Variation of the apparent rejection coefficients to lactose and protein.

VCF	Lactose (%)	Protein (%)
1.07	34	70
1.09	23	88
1.12	18	90
1.14	17	95
1.32	18	98

Acknowledgements

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References

[1] G. Daufin, J.P. Escudier, H. Carrére, S. Bérot, L. Fillaudeau, M. Decloux, Recent and emerging applications of membrane processes in the food and dairy industry, Food Bioprod. Process. Trans. Inst. Chem. Eng. Part C. 79 (2001) 89-102. doi:10.1016/S0960-3085(01)70244-1.

[2] P. Kumar, N. Sharma, R. Ranjan, S. Kumar, Z.F. Bhat, D.K. Jeong, Perspective of membrane technology in dairy industry: A review, Asian-Australasian J. Anim. Sci. 26 (2013) 1347-1358. doi:10.5713/ajas.2013.13082.

[3] G. Brans, C.G.P.H. Schroën, R.G.M. Van Der Sman, R.M. Boom, Membrane fractionation of milk: State of the art and challenges, J. Memb. Sci. 243 (2004) 263-272. doi:10.1016/j.memsci.2004.06.029.

[4] M. Cheryan, Ultrafiltration and Microfiltration Handbook., 1998. doi:97-62251.

[5] C. Baldasso, T.C. Barros, I.C. Tessaro, Concentration and purification of whey proteins by ultrafiltration, Desalination. 278 (2011) 381-386. doi:10.1016/j.desal.2011.05.055.

[6] E. El-Tanboly, M. El-Hofi, Khorshid, Recovery of cheese whey, a by-product from the dairy industry for use as an animal feed, J. Nutr. Heal. Food Eng. 6 (2017) 148-154. doi:10.15406/JNHFE.2017.6.00215.

[7] C. Mollea, L. Marmo, F. Bosco, Valorisation of Cheese Whey, a By-Product from the Dairy Industry, in: Food Ind., 2013. doi:10.5772/53159.

[8] F. V. Kosikowski, Whey Utilization and Whey Products, J. Dairy Sci. 62 (1979) 1149-1160. doi:10.3168/jds.S0022-0302(79)83389-5.

[9] F. Lipnizki, Cross-Flow Membrane Applications in the Food Industry, in: K. V. Peinemann, S.P. Nunes, L. Giorno (Eds.), Membr. Technol., Wiley-VCH, 2010: pp. 1–24. doi:10.1002/9783527631384.ch1.

[10] S.R. Cabral, A.P. Louro Martins, M. Norberta de Pinho, Optimization of the ultrafiltration in diafiltration mode to reduce the lactose content of protein concentrates, Universidade de Lisboa, 2015. https://fenix.tecnico.ulisboa.pt/cursos/meq/dissertacao/846778572210606 (accessed January 24, 2019).

[11] R. Kankanamge, C. Jeewanthi, N.K. Lee, S.K. Lee, Y.C. Yoon, H.D. Paik, Physicochemical characterization of hydrolysates of whey protein concentrates for their use in nutritional beverages, Food Sci. Biotechnol. 24 (2015) 1335-1340. doi:10.1007/s10068-015-0171-3.

[12] I. Jeli, R. Božani, L. Tratnik, Whey-based beverages- a new generation of diary products, Mljekarstvo. 58 (2008) 257-274. doi:http://www.scopus.com/inward/record.url?eid=2-s2.0-50449094791&partnerID=40&md5=974bda501a476372fe3f977781ce99a3.

[13] C.R. Shraddha RC, K.A. Nalawade T, Whey Based Beverage: Its Functionality, Formulations, Health Benefits and Applications, J. Food Process. Technol. 6 (2015) 495. doi:10.4172/2157-7110.1000495.

[14] A.R. Madureira, C.I. Pereira, A.M.P. Gomes, M.E. Pintado, F. Xavier Malcata, Bovine whey proteins - Overview on their main biological properties, Food Res. Int. 40 (2007) 1197-1211. doi:10.1016/j.foodres.2007.07.005.

[15] Z. Hafeez, C. Cakir-Kiefer, E. Roux, C. Perrin, L. Miclo, A. Dary-Mourot, Strategies of producing bioactive peptides from milk proteins to functionalize fermented milk products, Food Res. Int. 63 (2014) 71–80. doi:10.1016/j.foodres.2014.06.002.

[16] D.P. Mohanty, S. Mohapatra, S. Misra, P.S. Sahu, Milk derived bioactive peptides and their impact on human health – A review, Saudi J. Biol. Sci. 23 (2016) 577–583. doi:10.1016/j.sjbs.2015.06.005.

[17] S.F. Ahmadi, A. Nasirpour, S.A.H. Goli, E. Riahi, Effect of heat treatment and solution preparation procedure on colloidal stability of whey protein sour cherry beverage, Int. J. Dairy Technol. 71 (2018) 781-790. doi:10.1111/1471-0307.12498.

[18] B.C. Tranjan, A.G. Cruz, E.H.M. Walter, J.A.F. Faria, H.M.A. Bolini, M.R.L. Moura, L.M.J. Carvalho, Development of goat cheese whey-flavoured beverages, Int. J. Dairy Technol. 62 (2009) 438-443. doi:10.1111/j.1471-0307.2009.00491.x.

[19] J.M. Garrett, R.A. Stairs, R.G. Annett, Thermal Denaturation and Coagulation of Whey Proteins: Effect of Sugars, J. Dairy Sci. 71 (1988) 10-16. doi:10.3168/jds.S0022-0302(88)79518-1.

[20] R.E. Campbell, E.J. Kang, E. Bastian, M.A. Drake, The use of lactoperoxidase for the bleaching of fluid whey, J. Dairy Sci. 95 (2012) 2882-2890. doi:10.3168/jds.2011-5166.

[21] A. International, Official methods of analysis of AOAC International, 16th edition. Volume 2, 1995. AOAC (Association Off. Anal. Chem. Int. Arlington; USA. (1995).