

Optimization of the ultrafiltration in diafiltration mode to reduce the lactose content of protein concentrates

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Abstract

The main objective of this work is the separation and permeation of lactose present in whey previously concentrated by reverse osmosis, to be able to do beverage fortification trials in the future, with purified protein concentrates.

By the resistance model, it was found that the reversible resistance, which is associated with the concentration polarization and the material deposition on the membrane surface that is not chemically bonded to the membrane itself, is the one with the biggest contribution to the total resistance.

The process of fractionation by ultrafiltration of whey pre-concentrated by reverse osmosis was studied, measured in terms of permeation flow and apparent rejections. Rejections of 0.89 of protein and 0.55 of lactose were obtained.

A process of ultrafiltration in diafiltration mode was developed to obtain the protein fraction in the concentrated and the fraction rich in lactose in the permeate. Both the influences of the added amount of diavolume and of the frequency of diavolumes addition were studied, in order to compare the productivities obtained in terms of permeation flows and apparent rejection coefficients. In the UF in DF mode tests, the water addition allowed the permeation flow to increase and enhance the solutes passage which, according to the *MWCO*, should not be retained, in this case, to promote the lactose permeation.

Keywords: Whey pre-concentrated by reverse osmosis, Ultrafiltration/Diafiltration, Lactose, Protein, Rejection

1. Introduction

The whey is an aqueous portion that separates from the clot during the conventional production of cheese or casein manufacture [1]. This by-product of the dairy industry has a high

nutritional value, given by the presence of proteins with high essential amino acid content and relevant functional properties [2]. According to the literature, the whey can be classified as sweet or acid and its composition depends on

the type and method of manufacturing the cheese. These two types of whey are defined by reference to their final pH: acid whey has a pH \leq 5.1 while the sweet whey has a pH \geq 5.6 [3]. The typical composition of the sweet and acid whey is in Table 1.

Table 1 – Composition of sweet and acid bovine whey [4]

Parameter	Acid Whey (%m/m)	Sweet Whey (%m/m)
Total solids	6,5	6,4
Water	93,5	93,7
Fat content	0,1	0,5
Protein	0,8	0,8
Lactose	4,9	4,9
Ash	0,8	0,5

Due to the high organic load of the whey, it cannot be directly deposited on the soil or in water courses and, therefore, its reuse eliminates the environmental problem caused by the disposal of this effluent [5]. Thus, it can be inferred that not rational utilization of this by-product is an anti-environmental practice and consequently anti-economic.

The need to solve the problem of environmental impact, caused by the production of high volumes of this product, and the recognition of the nutritional value of whey made, from the sixties, new horizons for enhancement of this product open, with the development of membrane technologies. In fact, since that time, appeared a wide variety of new products extensively used by various industries, including the food industry.

In the food industry, whey can be used in its liquid form, protein concentrated, lactose concentrated or powdered. Approximately 50% of the world production of whey is treated and added to various food products, of which 45% is used in liquid form, 30% in the form of whey

powder, 15% as concentrated lactose and 10% as concentrated protein [6].

It is estimated that European production of whey, in 2013, was of 43 million tons [7]. On average, for the production of one kilogram of cheese ten liters of milk are required, with nine liters of whey recovering [5]. Thus, it is assumed that the generation of whey resulting from cheeses produced in Portugal is relevant. About 0.11 million tons in 2013 [7].

The works studying the production of whey derivative are crucial in order to explore new capabilities of using it, while reducing their environmental impact.

2. Experimental section

2.1 Materials

Whey. The whey used, previously concentrated by reverse osmosis, was donated by Queijo Saloio (Ponte de Rol, Torres Vedras, Portugal). This raw material comes from a cheese mix manufacture (milk from goat, cow and sheep). It was added an aqueous solution of hydrogen peroxide, commercially known as oxygenated water, to the whey for preserving it [8]. Subsequently, the raw material was cooled at 4 °C, in a refrigeration chamber.

Membranes. Commercial ultrafiltration membranes GR95PP, supplied by Alfa Laval Denmark, were tested. These membranes were characterized in terms of pure water hydraulic permeability, L_p , in terms of apparent rejections of reference solutes and in terms of molecular weight cutoff (*MWCO*). 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 L_p is the value

of the slope of the linear variation of pure water flux vs the transmembrane pressure. The *MWCO* calculation is based on the results of permeation experiments of solutions of reference solutes (polyethylenoglycols of 1, 4, and 6 kDa) with a concentration of 500 mg/L. The *MWCO* is obtained by the intersection of the curve of $\log(f/(1-f))$ vs the solute molecular weight with the 91% rejection line that corresponds to a value of $\log(f/(1-f))$ of 1. The solute concentrations are determined through total organic concentration (TOC) measurements. The information related to the characterization of the membrane is shown in Table 2.

Table 2 - Characteristics of GR95PP membranes

Membrane	Gr95PP
Material	Polyethersulphone
L_p (L/(h · m ² · bar))	1,21
<i>MWCO</i> (kDa)	7,5
Rejections (<i>f</i>) to reference solutes, %	
NaCl	13
CaCl ₂	0,47
Na ₂ SO ₄	70
Lactose	18

2.2 Procedure

Permeation Experiments. *Lab-Unit M20*, represented in Figure 1, was used in the permeation experiments with the membrane GR95PP with a surface area of 0.072 m². In order to eliminate fluctuations and assure reproducibility in the permeation essays, the membranes were subjected to compaction through permeation of pure water at a pressure 20% higher than the maximum operating pressure to be used and for a period of 3 hours [9].

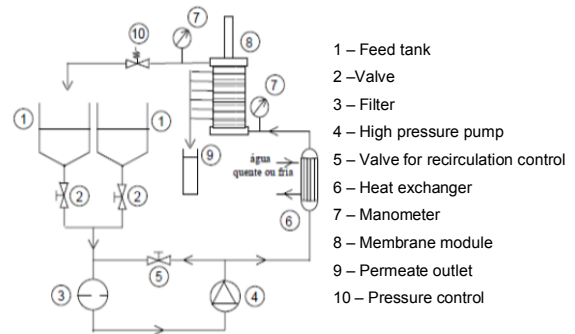


Figure 1 - Plate and frame DSS *Lab-Unit M20*

The permeation of reference solutions (polyethylenoglycols) for the membrane characterization was performed at a transmembrane pressure of 4 bar and using the maximum circulation flow.

To evaluate the influence of the pressure in the permeation flux, an experimental trial was conducted at different pressures (1 - 40 bar). In this trial, both the permeate and the retentate were maintained in complete recirculation.

The permeation experiment of whey pre-concentrated by reverse osmosis in concentration mode was performed at the transmembrane pressure of 12 bar. The volumetric concentration factor, VCF, is defined as the ratio of the initial volume to the final volume of whey in the feed tank. The VCF was varied from 1 up to 2.

The permeation experiments of whey pre-concentrated by reverse osmosis in UF/DF mode were performed at the transmembrane pressure of 12 bar. The volumetric concentration factor, VCF, is now defined as the initial volume of the feed plus the accumulated volumes of DF water added, divided by the final volume of whey in the feed tank.

In all the permeation experiments the stabilization time for each run was 30 minutes. This corresponds to the time needed to achieve a stable concentration in the permeate.

Membrane Cleaning. Membrane cleaning followed the permeation experiments, in total recirculation mode. This was performed with deionized water at 40 °C for at least 2 hours. In the case of that being ineffective (i.e., 8 hours after washing) an alkaline cleaning with Ultrasil 10, 0,1% for 15 minutes at 40 °C, would take place. The fluxes were compared with those measured before the experimental runs and the cleaning efficiency was assessed.

2.3 Analytical Methods

The physicochemical characterization of the whey samples, the concentrated and the permeated obtained from the UF and UF / DF, was based on the Portuguese standards for milk, since there are no specific standards for the characterization of whey.

About the samples, the following determinations were made: pH (Metrohm pH meter); total solids, according to NP – 475 (1983) procedure; fat content in the whey was determined using Geber's butyrometric determination, according to NP – 469 (1983) procedure; protein by the Kjeldahl method, according to NP – 1986 (1991) procedure; ash content according to NP – 477 (1983) and lactose through the balance:

$$Lactose = Total\ solids - Ash - Protein - Fat\ content \quad (1)$$

3. Theory

3.1 The resistance in series model [10,11]

The variation of the ultrafiltration permeation flux, J , as a function of the transmembrane pressure is then given by:

$$J = \frac{\Delta P}{\mu \cdot R_t} \quad (2)$$

where μ is the dynamic viscosity of the permeate and R_t is the total resistance. It is considered that the total resistance results from the intrinsic resistance of the membrane itself (R_m), the reversible fouling resistance (R_{Rev}) and the resistance to irreversible fouling (R_{Irrev}) Eq. (3).

$$R_t = R_m + R_{Rev} + R_{Irrev} \quad (3)$$

The membrane resistance corresponds to the resistance the membrane offers to the passage of pure water:

$$J_w = \frac{L_p}{\mu_w} \cdot \Delta P = \frac{\Delta P}{\mu_w \cdot R_m} \quad (4)$$

where μ_w corresponds to the viscosity of pure water at 25 °C. The viscosity of the water considered in the calculations was $1,003 \times 10^{-3}$ Pa.s [12].

The total resistance, R_t , is obtained from the permeation flow of the whey:

$$J_p = \frac{\Delta P}{\mu_p \cdot R_t} \quad (5)$$

where μ_p corresponds to the permeate viscosity, which was taken as equal to the viscosity of water at 25 °C.

The resistance to the reversible fouling is due to the concentration polarization and to the accumulation of solutes on the membrane surface that are not chemically bound to it. The resistance to the irreversible fouling is caused by compounds absorbed on the surface or within the pores of the membrane lying chemically bound to it.

At the end of whey ultrafiltration trial water is circulated at room temperature tangentially to the membrane at a low pressure (approximately 1 bar) and a maximum circulation flow. After

washing, the permeation flux, J_w^* , is determined using pure water.

$$J_w^* = \frac{\Delta P}{\mu_w \cdot R_t^*} \quad (6)$$

where R_t^* is the total resistance after washing and corresponds to:

$$R_t^* = R_m + R_{Irrev} \quad (7)$$

The value of the reversible resistance, R_{Rev} , is obtained by the difference between total resistance, R_t and the sum of R_m with R_{Irrev} .

3.2 Sizing equations in batch mode [13]

The variation of feed volume, V , over time, t , is due to the permeate outlet, J , through the permeation area, A , which is described by:

$$-\frac{dV}{dt} = J \cdot A \quad (8)$$

The balance to the solute leads to the following equation:

$$-\frac{d(VC_r)}{dt} = J \cdot A \cdot C_p \quad (9)$$

where C_r and C_p are the solute concentration in the concentrated and in the permeate, respectively. In pressure driven membrane processes is reasonable to assume that the rejection coefficient, f , is independent from the concentration factor. Assuming this hypothesis and taking into account the previously presented equations one can obtain:

$$V \frac{dC_r}{dV} = f \cdot C_r \quad (10)$$

The separation and integration between $V = V_o$ and $V = V_f$ from Eq. (10) leads to the following expression:

$$C_r = C_a \cdot \left(\frac{V_o}{V_f}\right)^f \quad (11)$$

That can also be described by:

$$C_r = C_a \cdot (FCV)^f \quad (12)$$

where C_a is the solute concentration in the feed, V_o is the initial volume of the feed and V_f is the concentrate final volume.

4. Results and discussion

4.1 Characterization of whey pre-concentrated by osmosis

The whey concentrated by reverse osmosis is sweet (pH close to 6.0) for what its composition should be compared with the sweet bovine whey, showed in Table 1.

Table 3 – Typical composition of whey pre-concentrated by reverse osmosis

Parameter	Content
pH	6,07
Total solids (g/100g)	16,72
Fat content (g/100g)	0,30
Protein (g/100g)	2,00
Lactose (g/100g)	13,08
Ash (g/100g)	1,34

The whey concentrated by reverse osmosis is richer in total solids (16,72 g/100g) when compared to the bovine whey (6,4 g/100g), mainly because of its richness in crude protein (2,00 g/100g compared to 0,8 g/100g sweet bovine whey) and lactose. The lactose content obtained for this whey, 13,08 g/100g, is much higher than the typical value of 4,9 g/100g, present in the literature. These results can be

explained by the fact that the reverse osmosis process allows only the passage of water and certain salts. When the OI process, the whey constituents such as lactose, protein and fat are rejected, making the whey more concentrated, this being the most obvious effect from the results obtained.

Alongside this, the fact that this whey is not only from cow's milk, but a mixture of cow, goat and sheep is another reason that explains the big difference between the typical values of bovine whey and the ones presented. These differences may still be related or with the composition of the original milk and/or with the processes of making cheese, from which the whey resulted.

4.2 Experiments in total recirculation mode

In order to study the influence of the transmembrane pressure on the permeation fluxes of UF of whey pre-concentrated by reverse osmosis, experiments in total recirculation mode were carried out at the maximum recirculation speed.

Figure 2 displays the variation of permeation fluxes with transmembrane pressure. It is found that the permeation flow increases with the applied transmembrane pressure and that the relationship between the permeation flow of the whey and the transmembrane pressure is linear up to 10-12 bar. From this value and up to 30 bar there is a deviation to the linearity. From 30 bar, it reaches a limit of $8,79 \text{ L}/(\text{h} \cdot \text{m}^2)$.

It is observed that the whey permeation flows are always lower, in the examined pressure range, and deviate increasingly from the corresponding pure water flows as the pressure increases, indicating that there are resistances to the mass transfer beyond the intrinsic resistance of the membrane, which is $2,97 \times 10^{14} \text{ m}^{-1}$. The deviation to the pure water permeation flow is a result of the resistance to the reversible resistance ($4,27 \times 10^{14} \text{ m}^{-1}$), which is due to concentration polarization and accumulation of solutes on the membrane surface that are not chemically bonded to it, and the irreversible resistance ($3,18 \times 10^{13} \text{ m}^{-1}$), which corresponds to the adsorption of compounds on the surface or within the membrane pores and that are chemically bounded to its material.

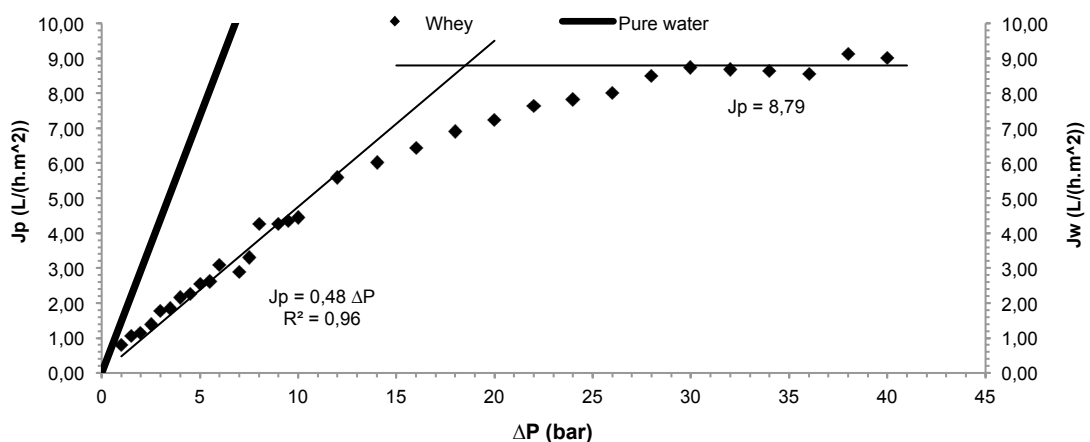


Figure 2 –Variation of permeation flux of whey pre-concentrated by reverse osmosis (J_p) and pure water (J_w) with transmembrane pressure. Installation *Lab-Unit M20*- Membrane: GR95PP; Membrane surface area: $0,072 \text{ m}^2$; Temperature: $25 \text{ }^\circ\text{C}$; Maximum circulation speed

4.3 Experiments in concentration mode

The evaluation of the permeation flux in concentration mode at the pressure of 12 bar is displayed in Figure 3. For this test 5L of whey pre-concentrated by reverse osmosis are used, concentrating to a FCV of 2,00.

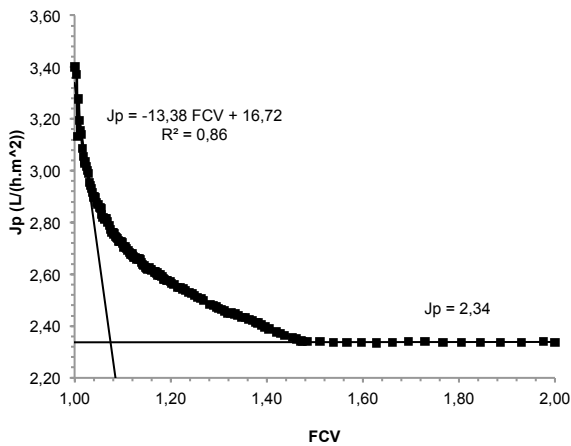


Figure 3 –Variation of permeation flux of whey pre-concentrated by reverse osmosis (J_p) with volumetric concentration factor (VCF).

Installation *Lab-Unit M20*- Membrane: GR95PP; Membrane surface area: 0,072 m²; Pressure: 12 bar; Temperature: 25 °C; Maximum circulation speed

There was a decrease in permeation flux with increasing FCV, which stabilizes at a constant value (2,34 L/(h.m²)) starting at a volumetric concentration factor of around 1,50. This permeation flows decrease with the concentration factor in volume is a characteristic of tests in concentration mode and is a consequence of the phenomena of concentration polarization and/or fouling of the membranes, which intensity increases with the concentration.

The protein and lactose apparent rejection coefficient, for the concentration factor of 2,00, was 89% and 55%, respectively. These values indicate that the membrane has a high selective capacity for the protein, occurring almost

complete retention, and that lactose is incorporated in the solution as the concentrations of the other increases during the test.

4.4 Experiments in diafiltration mode

Preliminary assessment of diafiltration

During the test 20% of pure water was added, in relation to the volume observed in the feed tank, when a decrease of 16% and 4% of the permeation flow was observed, which correspond to a FCV of 1,05 and 1,25, respectively. Figure 4 represents the evolution of the permeation flow and the feed volume with time.

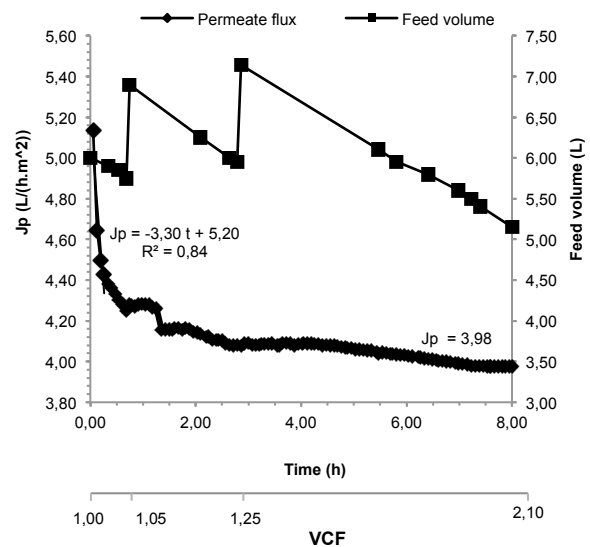


Figure 4 – Variation of permeation flux of whey pre-concentrated by reverse osmosis (J_p) and the feed volume with time.

Installation *Lab-Unit M20*- Membrane: GR95PP; Membrane surface area: 0,072 m²; Pressure: 12 bar; Temperature: 25 °C; maximum circulation speed

The dilution of whey causes an increase of the permeation flow in the first instant of the concentration step. During the test it is found that the flow decreases sharply to a low volumetric concentration factor. The variation of

the whey permeation flux over time shows an asymptotic linear behaviour until 0,236 h where $J_p = -3,30 \cdot t + 5,20$. Although the water additions do not allow the recovery of the flux initially obtained, these successive water additions allow the flow to not decrease significantly during the test.

The variation of the apparent rejection coefficients of lactose and protein with the volumetric concentration factor (VCF) is shown in Table 4.

Table 4 – Variation of the apparent rejection ratio of 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 concentration factor. It can be concluded that the diafiltration increased lactose permeation, reducing the retention of this solute without compromising the selectivity of protein by the membrane.

Optimization of UF/DF

✓ *Influence of the quantity of added diavolume*

Initially the pre-dilution of the whey pre-concentrated by reverse osmosis was done, by

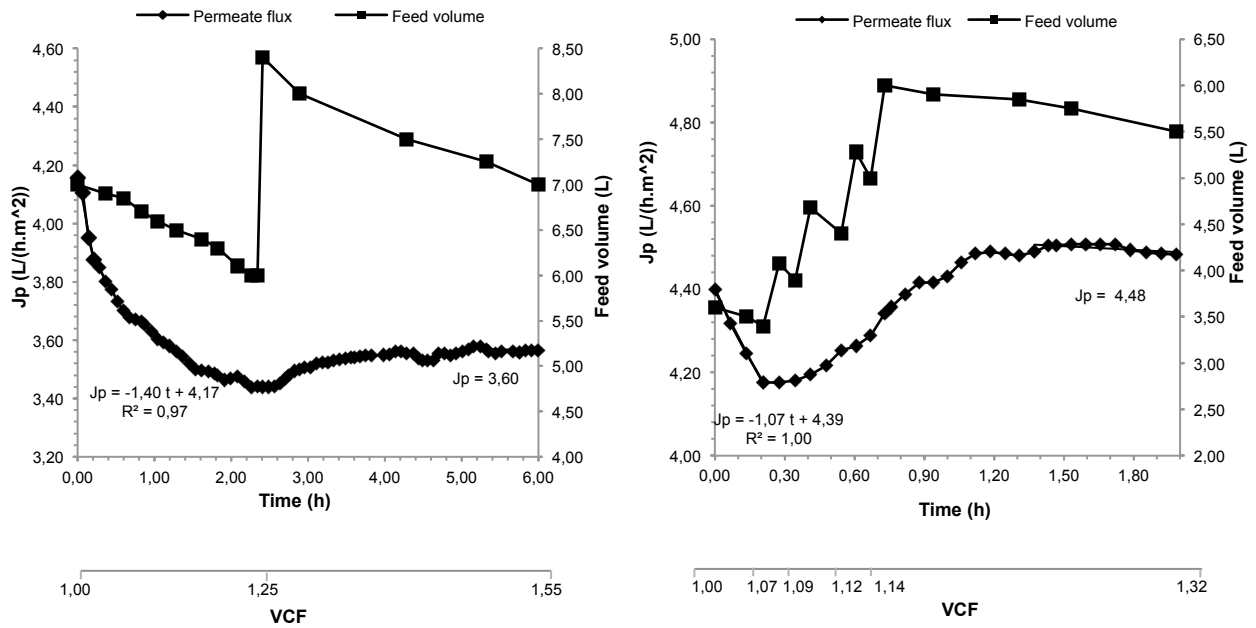
adding 40% of pure water relative to the volume present in the feed tank. During the test 40% of pure water was added for a 16% decrease of the permeation flow, which corresponds to a FCV of 1,25 (Figure 5).

Figure 5 shows that the permeation flow variation of whey over time has a linear asymptotic behaviour until 0,233h, where $J_p = -1,40 \cdot t + 4,17$. It is observed that, for the same time interval, the slope of J_p of this test as a function of time is less than the slope observed for the linear relationship of the preliminary evaluation test of the UF/DF. When compared to the initial flow obtained in the preliminary evaluation test of UF/DF, the initial permeation flow obtained from this test is lower. This is related to the composition of the whey used in these two tests. The variation in the apparent rejection coefficients of the lactose and protein as a volumetric concentration factor (VCF) is shown in Table 5.

Table 5 – Variation of the apparent rejection ratio of lactose and protein

VCF	Lactose (%)	Protein (%)
1,25	18	56
1,55	11	73

It was concluded that adding a larger quantity of pure water causes a decrease in the protein apparent rejection, when compared with the preliminary UF/DF test, which translates into a loss of this macromolecule to the permeate.



Figures 5 e 6 - Variation of permeation flux of whey pre-concentrated by reverse osmosis (J_p) and the feed volume with time. Influence of the quantity of added diavolume (left) and Influence of the volume adding rate of the diavolume (right)

Installation *Lab-Unit M20*- Membrane: GR95PP; Membrane surface area: 0,072 m²; Pressure: 12 bar; Temperature: 25 °C; maximum circulation speed

✓ *Influence of the volume adding rate of the diavolume*

Initially the dilution of the whey pre-concentrated by reverse osmosis was done, by adding 20% of pure water relative to the volume present in the feed tank. During the test, 20% of pure water relative to the volume observed in the feed tank was added 10, 20, 30 and 40 minutes after the beginning of the test (Figure 6). Successive additions of water in a short period of time, not only interrupt the decrease of permeation flow but also promote its increase. The variation of the whey permeation flow over time shows a linear asymptotic behavior until 0,210h, where $J_p = -1,07 \cdot t + 4,39$. It is concluded that the decrease of permeation flow in this test is significantly lower than the decrease observed in the UF/DF preliminary evaluation test, for a similar time interval. When compared to the initial flow obtained in the

UF/DF preliminary evaluation test, the initial permeation flow obtained in this test is lower. This is related to the composition of the whey used in these two tests.

The apparent rejection coefficients variation of the lactose and protein as a volumetric concentration factor (VCF) is shown in Table 6.

Table 6 – Variation of the apparent rejection ratio of 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

It is verified that the rejection coefficient to the protein rises as lactose rejection decreases with increasing FCV. Successive additions of pure water, in short time intervals, allow to get

high protein rejections and increasing, which indicates that the retention to this solute is almost total.

5. Conclusions

Throughout this work a process of ultrafiltration in diafiltration mode was developed and optimized to obtain the protein fraction in the concentrated and the permeate fraction rich in lactose in the permeated. In these tests, it was found that the addition of pure water increased the permeation flow and enhanced the passage of solutes, which, according to the *MWCO*, should not be retained, or by other words, promote the lactose permeation.

It was found that the total resistance to mass transfer is $7,55 \times 10^{14} \text{ m}^{-1}$ for the whey pre-concentrated by reverse osmosis, coming the largest contribution to this resistance from the reversible resistance ($4,27 \times 10^{14} \text{ m}^{-1}$).

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