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Whey protein concentration by ultrafiltration and study of functional properties

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ABSTRACT: *This paper aim to evaluate the ultrafiltration (UF) process for constituents recovery from whey. Sequences of factorial designs were performed by varying temperature (5 to 40°C) and pressure (1 to 3 bar), to maximize the proteins concentration using membrane of 100kDa in dead end system. Based on the best result new experiments were performed with membrane of 50kDa and 10kDa. With the membrane of 50 the protein retention was about 3 times higher than the membrane of 100kDa. The concentrated obtained by UF membrane of 10kDa, 10°C and 2 bar in laboratory scale showed a mean protein retention of 80 %, greater protein solubility, emulsion stability and the identification of β-lactoglobulins (18.3 kDa) and α-lactalbumin fractions (14.2kDa). Therefore, the use of membrane of 100 and 50kDa are became a industrially recommendable alternatives to concentration of whey proteins, and/or as a previous step to the fractionation of whey constituents using membrane ≤10kDa, aiming at future applications in different areas (food, pharmaceutical, chemical, etc.).*
Key words: *ultrafiltration, whey, dead-end; solubility and emulsion stability.*

Concentração de proteínas de soro por ultrafiltração e estudo das propriedades funcionais

RESUMO: *O objetivo do estudo foi avaliar o processo de Ultrafiltração (UF) na recuperação dos constituintes do soro de leite. Planejamentos fatoriais sequenciais foram realizados, variando a temperatura (5 a 40°C), a pressão (1 a 3 bar) e visando maximizar a concentração de proteínas usando membrana de 100kDa em sistema dead end. Baseados nos melhores resultados, foram realizados experimentos com de 50kDa e 10kDa. Em relação a membrana de 50kDa, a retenção de proteínas foi cerca de três vezes maior em relação a membrana de 100kDa. O concentrado obtido por membrana UF de 10kDa, 10°C e 2 bar, em escala laboratorial, mostrou uma retenção média de proteína de 80%, maior solubilidade protéica, estabilidade da emulsão e a identificação das frações β-lactoglobulins (18.3kDa) e α-lactalbumin (14.2kDa). Portanto, o uso de membranas de 100 e 50kDa são alternativas recomendáveis industrialmente à concentração de proteínas de soro de leite, e/ou como etapa anterior ao fracionamento de constituintes do soro usando membrana ≤10kDa, visando aplicações futuras em difentes áreas (alimentícia, farmacêutica, química, etc).*
Palavras-chave: *Ultrafiltração, soro de leite, dead end, solubilidade, estabilidade de emulsão.*

INTRODUCTION

The whey represent about 85 to 90% of the total volume of milk used for cheese production, and contains approximately 55% of milk nutrients, including proteins, lactose, soluble vitamins and minerals (BRANDELLI et al., 2015; PALATNIK et al., 2015). However, if on one hand the whey is viewed as a pollution agent, on the other side, it can be regarded as a value-added product, rich in nutrients, especially proteins with high biological quality, with nutritional and functional properties (emulsifying, foaming and gelling), may be incorporated into a wide range of foods (ARUNKUMAR & ETZEL, 2015).

The protein concentration can improve the expression of their properties, allowing its use in different products in the trade. One alternative to recovery the whey constituents is to use membrane technology, where the ultrafiltration (UF) is the main process to fractionate whey constituents (BÁGUENA et al., 2015; WELSH et al., 2017).

In this context, the objective of the present study was to evaluate the UF process on recovery of milk whey constituents in laboratory scale, using a dead-end flow, with flat polysulfone amide membrane (10, 50 and 100kDa), evaluating permeate flows, physicochemical characteristics and functional property of the concentrates and permeates.

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MATERIALS AND METHODS

Ultrafiltration process

Bovine whey (in nature) was supplied by an industry of whey processing of the southern region of Brazil. The UF process (dead end) was conducted in laboratory scale. The system was composed of water bath with thermostat (Servilab, SE 100AG); nitrogen cylinder (analytical N2 5.0 - White Martins); UF membrane separation module (conventional flow, volume of 200mL); (d) Magnetic stirrer (ARE Heating Magnetic Stirrer- Scientific Velp). Three flat membranes were used in the work: one of 100kDa, of polysulfone amide (GE-Osmonics (Sepa MW), one of 50kDa and one of 10kDa of polyethersulfone (Microdyn-Nadir GmbH), with permeation area of 0.001134m². To evaluate the effects of temperature and pressure under the permeate and concentrate flow of the 100kDa membrane, a factorial design 2^2 (design 1) was first carried out, varying the temperature (20 to 40°C) and the pressure (1 to 3 bar) for water and milk whey.

The levels of the variables were defined based on literature information (BALDASSO et al., 2016; BARUKCIC et al., 2014) and on preliminary tests. According to the results obtained in the Design 1, a new factorial design 2^2 (Design 2) was carried out, varying the temperature (5 to 15°C) and using the same pressure (1 to 3 bar). From the maximized condition obtained of the membrane of 100kDa, were performed experiments with the membrane of 50kDa and 10kDa. The dependent variables were the permeate flow of water and whey, total protein content, amount of solids, conductivity, acidity, pH, lactose and total minerals in the concentrate and permeate.

The permeate flow (J_m) were performed by the measurement of permeate collected in a graduated glass, calculated by the permeate volume (L) in relation to the permeation area (m^2) and the time (h). The water membrane resistance (Rm) and milk whey resistance (Rt) were determined by Darcy's law for the membrane of 100kDa. The whey viscosity at temperatures of (15 and 40°C) was determined using a Brookfield Mark rotational viscometer (PROGRAMMABLE DV-III + Rheometer). The water viscosity data at temperatures of 15 and 40°C were taken from SHAMES (1995).

The rejection coefficient was calculated for protein, total solids, lactose and minerals by the relation between the concentration in the feed and its concentration in the permeate.

The rejection coefficient (R) was calculated according to equation 1 (PABBY et al., 2015).

$$R = \left(\frac{c_r - c_p}{c_r}\right) \times 100 = \left(1 - \frac{c_p}{c_r}\right) \times 100 \quad (1)$$

Where: c_r represents the concentration of a solute in the retentate and c_p is the concentration of the same solute in the permeate, and the rejection is presented in %.

Physico-chemical characterization

The in nature whey and the fractions of concentrates and permeates obtained from the UF processes were analyzed in relation to total protein - Kjeldahl method (AOAC, 2005); separation of protein fractions and identification of molecular weight by electrophoresis according to LAEMMLI (1970); total solids by the gravimetric method in a recirculation oven (Fanem - model 320 - SE) at 105ºC until constant weight (BRASIL, 2005); electrical conductivity using a conductivity meter (RS 232-METER 8306); total acidity was expressed in Dornic (AOAC, 2005); pH by potentiometric method (AOAC, 2005); lactose by the DNS method (3,5-dinitro salicylate) according to methodology described by MILLER (1959); total minerals was determined according to the methodology described by BRASIL (2005).

The protein soluble was measured of the concentrate obtained by UF (50 and 10kDa) lyophilized, where the sample was determined by dispersing in 10mM sodium phosphate buffer (pH 7.0) and stirring for 30min at room temperature to prepare a 0.1% w/v solution. The dispersions were then centrifuged at 10,000 *x* g for 10min at 4°C and the protein content of the supernatant was determined by the Lowry method (LOWRY et al., 1951).

Emulsion preparation

The emulsions were prepared with and oil: water (o/w) ratio of 25:75 v/v. The aqueous phase was a solution of soluble proteins of 1mg/mL concentration in phosphate buffer 0.01M pH 7.0. The oil used was commercial sunflower oil. The emulsions were done by mixing the aqueous and oil phases with an Ultraturrax homogenizer (IKA-Labortechnik) at 20,000rpm during 1min.

Creaming stability

Immediately after homogenization creaming stability of the o/w emulsions were optically characterized using a vertical scan analyzer (Turbiscan Classic MA2000, Formulation. Toulouse, France). Curves of backscattering (% BS) and transmission percentages as a function of time and tube length were obtained.

The studies of creaming and destabilization of the emulsions were made by measuring retrodispersion

once a minute for 60min. The kinetics of destabilization was determined at a tube length of 10-20mm according model developed by PANIZZOLO et al. (2014).

The measure of BS is linked to the concentration and average size of the emulsion droplets as a function of the height measuring device (ABISMAÏL et al., 2000). From the profiles obtained the mean values for % BS were calculated in the 50-53mm zone (% BS-50-53), corresponding to the cream part of the tube. To measure the global stability of the emulsions after 24h of storage, the destabilization percentage (% D) was defined by PALAZOLO et al. (2005).

Statistical analysis

The results obtained from the experimental design where used to evaluated the differences between means variance analysis (ANOVA) follow by Tukey´s test and/or t student using *Statistic* software, version 5.0, at a significance level of 95% of confidence.

RESULTS AND DISCUSSION

Effects of pressure and temperature on the membrane of 100kDa

Table 1 shows the matrix of the factorial design 2^2 (real and coded values) - Design 1, and values of mean permeate flow (L m^{-2} h^{-1}) for water and whey, obtained from the UF process with membrane of 100kDa. For water high flows (Table 1) were verified in the tests 5, 6 and 7 (Design 1) which was operated at 30°C and 2 bar. While for the whey the highest flow was 24.82 L/m^2 h at 40°C and 3 bar (test 4 - Design 1) and the lowest was 14.17Lm^{-2} h^{-1} at 5°C and 1 bar (test 1 - Design 2).

Equations 2 and 3 shows a first order coded model for whey flow as a function of temperature and pressure, for the designs 1 and 2, within the ranges studied. The models were validated by variance analysis (ANOVA). The correlation coefficients were 0.95 and 0.91, and the results were significative ($p<0.05$), allowing the construction of the contour curves (Figure 1).

$$FMS=21.25+1.05X_1+3.65X_2 \quad (2)$$

$$FMS=17.02+0.64X_1+1.64X_2 \quad (3)$$

Where FMS is the whey flow (L/m^2 h), X_1 is the temperature (°C) and X_2 is the pressure (bar).

The larger mean whey permeates flows were obtained at high pressures and temperatures, being recommended ranges of pressures greater than 2 bar and temperatures above 10°C. These temperatures seem to be more suitable, since no acidification was verified in the concentrates (Table 2). Low temperatures may have additional benefits in the filtration which reducing on the denaturation of whey proteins, reducing on the membrane fouling caused by calcium phosphates and reduce the growth of thermophilic bacteria. However, lower temperatures reduce diffusivity, and hence mass

Table 1 - Matrix of the factorial design 2^2 (real and coded values) - Designs 1 and 2 and values of permeate flow (Lm^{-2} h^{-1}) for water and whey using membrane of 100 kDa.

Tests	Independent variables*		Permeate flow (L m^{-2} h^{-1})	
	X_1	X_2	Water	Whey
Design 1				
1	20 (-1)	1.0 (-1)	120.33	15.41
2	40 (1)	1.0 (-1)	165.68	18.17
3	20 (-1)	3.0 (1)	389.05	23.35
4	40 (1)	3.0 (1)	431.92	24.82
5	30 (0)	2.0 (0)	440.39	22.17
6	30 (0)	2.0 (0)	433.51	22. 29
7	30 (0)	2.0 (0)	443.57	22.53
Design 2				
1	5 (-1)	1.0 (-1)	114.86	14.17
2	15 (1)	1.0 (-1)	170.97	15.53
3	5 (-1)	3.0 (1)	392.22	17.53
4	15 (1)	3.0 (1)	434.04	18.73
5	10 (0)	2.0 (0)	226.02	17.70
6	10 (0)	2.0 (0)	230.78	17.73
7	10 (0)	2.0 (0)	222.84	17.76

*X_1= Temperature (°C); X_2= Pressure (bar). Fixed dependent variables: feed volume (200mL).

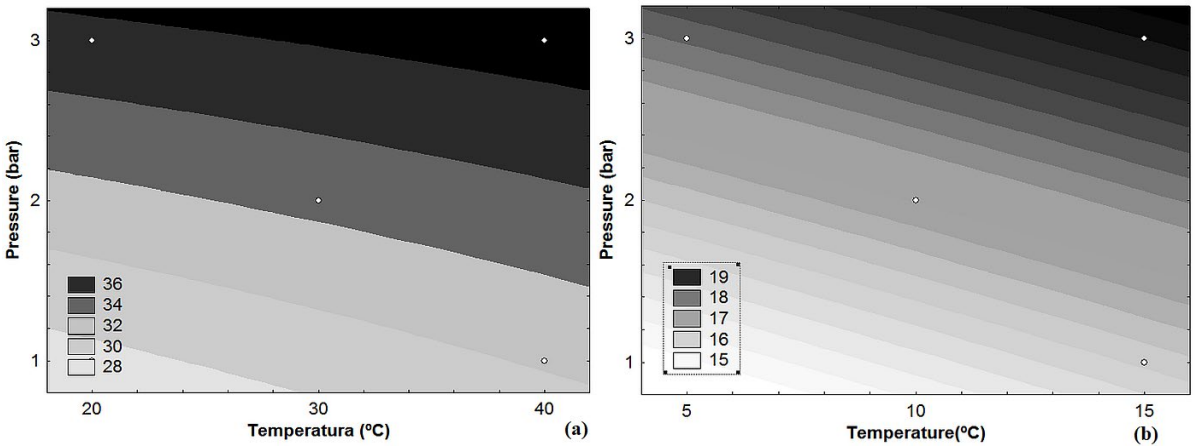

Figure 1 - Contour curves for whey flow (L/m²h), obtained in the Design 1 (a) and 2 (b), respectively.

transfer coefficient, resulting in flow rates lower than UF performed at high temperatures, regardless of the membrane pore size.

Although the contour curves (Figure 1) indicate largest permeate flows at 3 bar, caution is required in setting the operating pressure. Whey in this system with high pressures intensified the polarization phenomena by concentration and fouling of the membrane, resulting in permeate flow similar to those obtained at 2 bar. So the pressure of 2 bar appears to be more suitable for whey UF in this system.

The permeate flow for water (Figure 2A-C) showed almost stationary profile over the time for all tests, and this behavior is already expected since the fluid used (deionized water) is free of contaminants (suspended and dissolved material). In the whey flow (Figure 2B-D) for all tests we can observed that there was a decrease in the permeate flow over the operating time, this fall is mainly due to polarization phenomena of concentration and fouling. It occurred since the components of the whey, mainly the proteins, are retained and accumulate on the membrane surface forming a barrier. Another factor that can decrease the flow is the type of flow (conventional) since has a greater concentration of particles next to the membrane with the time, causing a greater fall in the permeate flow due to the increase of the filtration resistance due to the increase of cake resistance.

The permeate flow of whey (Figure 2B-D), test 1 and 2 at 1 bar presented lower flow than the other test, and the tests 3 and 4, and 5 6 and 7 which operated at 3 and 2 bar respectively not showed differences.

Table 2 - Matrix of the factorial design 2^2 (real and coded values) - Design 1 and response in terms of pH and acidity (°D) for the concentrates and permeates obtained after UF.

Tests	---Independent variables*----		----------pH----------		----------Acidity** (°D)----------	
	X_1	X_2	Concentrate	Permeate	Concentrate	Permeate
1	20 (-1)	1.0 (-1)	5.00^b (±0.01)	5.12^a (±0.03)	27.10^a (±0.85)	22.83^b (±0.29)
2	40 (1)	1.0 (-1)	4.37^b (±0.03)	4.49^a (±0.01)	31.73^a (±0.64)	28.50^b (±0.50)
3	20 (-1)	3.0 (1)	5.14^b (±0.03)	5.20^a (±0.03)	26.30^a (±0.20)	23.03^b (±0.06)
4	40 (1)	3.0 (1)	4.40^b (±0.01)	4.51^a (±0.01)	30.33^a (±0.29)	27.67^b (±0.58)
5	30 (0)	2.0 (0)	4.66^b (±0.01)	5.01^a (±0.03)	26.43^a (±0.51)	23.17^b (±0.29)
6	30 (0)	2.0 (0)	4.65^b (±0.01)	4.98^a (±0.01)	26.50^a (±0.50)	23.50^b (±0.50)
7	30 (0)	2.0 (0)	4.64^b (±0.01)	5.0^a (±0.02)	26.33^a (±0.58)	23.33^b (±0.58)

*X_1= Temperature (°C); X_2= Pressure (bar); **mean (± standard deviations) followed by same letters on lines represents no significant difference at 5% level (t student test). Fixed independent variables: feed volume (200mL), UF time of 90min.

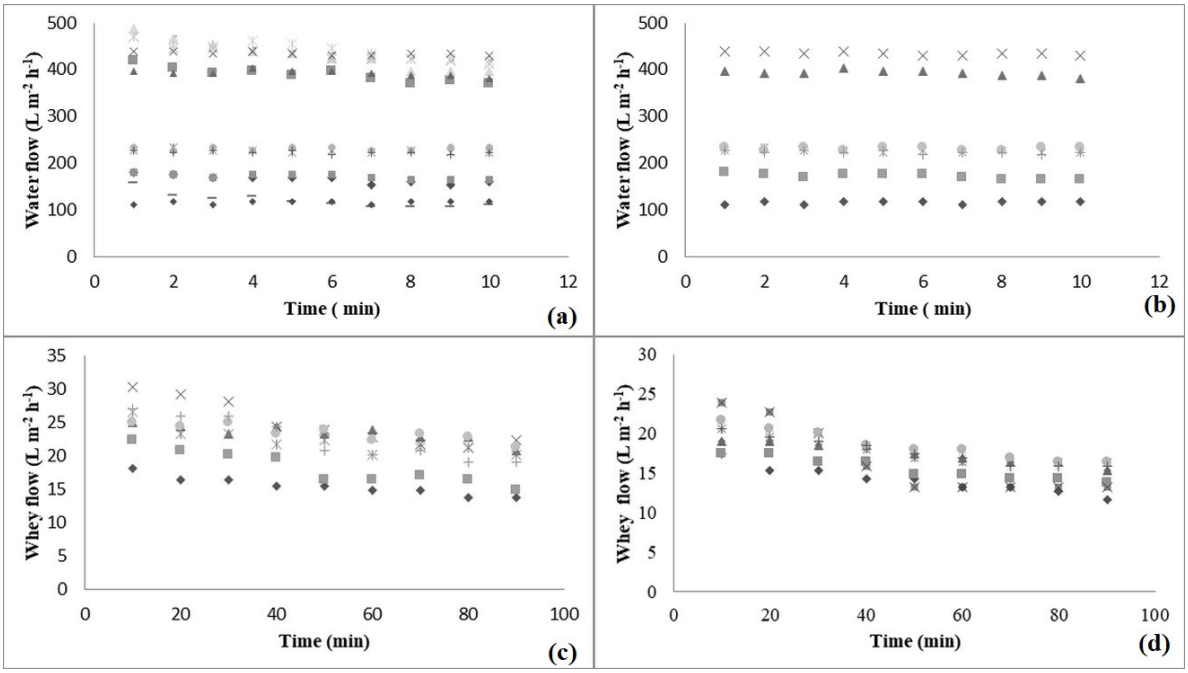

Figure 2 - Permeate flows of water ("a" and "c") and whey ("b" and "d") as a function of time using a membrane of 100 kDa in the Design 1 and 2, respectively.

Possibly for this process at 2 bar is the limit flow, which is reached when the pressure increase in the system no influences in the flow increase, where the flow remains practically constant independent of the increase in pressure. In this case the protein molecules are deposited on the membrane surface, forming a gel layer, causing the phenomena of fouling and polarization by concentration, and this layer causes an additional resistance to permeate flow.

At high temperatures the whey viscosity is reduced, increasing the fluid speed directed to the membrane, in this way increasing its initial permeation and the drag of solutes towards the membrane, accelerating the concentration polarization.

The UF membrane resistance using whey at 40°C (viscosity of $1.79x10^{-3}$kg m^{-1} s^{-1}) was $2.43x10^{7}$ m^{-1}, around 6.34 times higher than obtained for water. While, at 15°C (viscosity of $2.07x10^{-3}$ kg m^{-1} s^{-1}) the resistance of the whey was $2.77x10^{7}$ m^{-1}, being 12.70 times higher than that found for water. The highest resistance observed for whey in relation to water at both temperatures is given as a function of the solids present in the solution that difficult the membrane cross, besides the whey viscosity is higher than the water, making it difficult to cross the membrane.

The *in nature* whey had an average composition: 5.6% total solids, 4.66% lactose, 0.91% protein, 0.6% total minerals, pH of 6.49 and acidity of 12.20ºD. In relation to pH and acidity the whey can be classified as sweet whey (MACEDO et al., 2015).

Table 2 shows the matrix of factorial design 2^2 (real and coded values) - Design 1 the response in terms of pH and acidity (°D) for the concentrate and permeate obtained after the UF using flat membrane of 100 KDa. Both permeate and concentrate shows an increase in the acidity compared with the *in nature* whey (12.20°D), possibly due of the bacteria action during the remained of the whey in the UF system. The acid values for the concentrates were higher ($p<0.05$) than those found for permeate in all the tests. The highest acidity levels (31.73 and 30.33°D) were observed in concentrates at 40°C (tests 2 and 4).

The increase in acidity levels observed in Design 1 (Table 2) occurred due to lactose degradation (data not shown) and lactic acid production due to the action of lactic acid bacteria present in the whey, because the temperatures 20-40°C are considered optimal for its development. This could be seen by the Pareto chart (Figure 3A) were the acidity was positively influenced ($P<0.05$) by the temperature. Similarly, in both fractions (permeated and concentrated) a decrease in pH occurred in relation to *in nature* milk (mean pH 6.49). The lower pH values for permeates (4.49) and the concentrates (4.37) were obtained at 40°C.

Based on concentrates results a new factorial design (Design 2) was conducted for UF with a flat

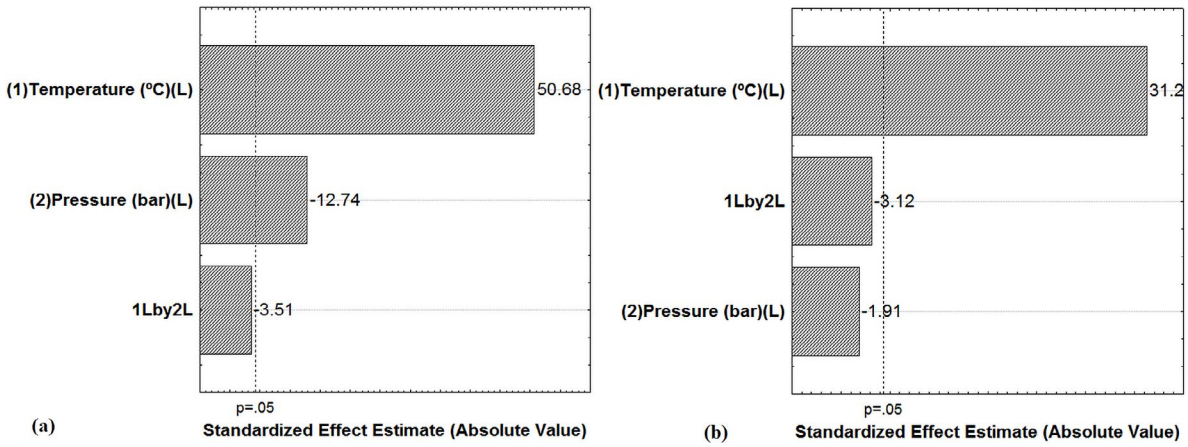

Figure 3 - Pareto chart with the estimated effect (absolute value) of acidity (°Dornic) for the concentrate (a) and permeate (b) obtained after the UF process of factorial design 2^2 – Design 1, respectivelly.

membrane of 100 kDa, where the temperature levels were 5, 10 and 15°C and the pressures were maintained at the same levels of the Design 1 (Table 3) due to system limitation. No significant difference ($P > 0.05$) was observed between the acidity and pH of permeate and concentrate in the experiments, and the acidity values were similar to those obtained in the *in nature* whey (12.20°D). For the permeated fractions the pH presented a variation between 6.44 and 6.50, while the concentrated fractions presented slightly lower values ($P < 0.05$) in all the tests ranging from 6.33 to 6.42.

Equations 4, 5 and 6 shows the first order coded models for acidity, pH and solids rejection, respectively, of the concentrates as a function of temperature (significate variables $P<0.05$). The model was validated by variance analysis (ANOVA), with correlation coefficient of 0.95, 0.92 and 0.99, and the results were significate ($P < 0.05$), allowing

Table 3 - Tests the factorial design 2^2 (real and coded values) - Design 2 and response in terms of pH, acidity (°D), conductivity and rejection coefficient (protein, solids, lactose and minerals) for the concentrates and permeates obtained after UF.

Test	----------pH*----------		--------Acidity* (°D)--------		-Conductivity (mS cm^{-1})*--		----Rejection Coefficient (%)*-----			
	Concentrate	Permeate	Concentrate	Permeate	Concentrate	Permeate	P.	S.	L.	M.
1	6.41^b (±0.01)	6.50^a (±0.02)	12.33^a (±0.58)	12.17^a (±0.76)	5.12^b (±0.02)	5.22^a (±0.03)	70.33	21.79	4.72	20.00
2	6.33^b (±0.01)	6.45^a (±0.03)	13.33^a (±1.15)	12.67^a (±0.50)	5.14^b (±0.03)	5.25^a (±0.05)	64.84	19.64	5.15	18.33
3	6.42^b (±0.03)	6.50^a (±0.04)	12.33^a (±0.29)	12.33^a (±0.58)	5.13^b (±0.05)	5.23^a (±0.03)	67.03	18.04	3.43	21.67
4	6.36^b (±0.03)	6.47^a (±0.01)	13.67^a (±0.58)	13.17^a (±0.29)	5.15^b (±0.04)	5.24^a (±0.04)	64.84	17.50	3.86	20.00
5	6.38^b (±0.01)	6.46^a (±0.03)	13.00^a (±1.00)	12.33^a (±0.58)	5.14^b (±0.04)	5.26^a (±0.03)	72.53	18.93	4.72	21.67
6	6.39^b (±0.02)	6.46^a (±0.02)	12.67^a (±0.51)	12.33^a (±0.58)	5.15^b (±0.03)	5.25^a (±0.05)	70.33	18.75	4.51	23.33
7	6.36^b (±0.02)	6.44^a (±0.03)	12.67^a (±0.58)	12.00^a (±1.00)	5.13^b (±0.04)	5.24^a (±0.04)	71.43	18.75	4.08	23.33

*mean (± standard deviations) followed by same letters on lines represents no significant difference at 5% level (t student test). Fixed independent variables: feed volume (200mL), UF time of 90min. P= protein; S=solids; lactose; M= minerals.

the construction of the contour curves (Figure 4).

$ACID_C=12.856+0.598X_1$ (4)

$pH_C=6.377-0.368X_1$ (5)

$R_{st}=6.09-0.67X_1-1.47X_2+0.4X_1X_2$ (6)

Where $ACID_c$ is the concentrate acidity (°D), pH_c is the concentrate pH, R_{st} is the rejection coefficient, X_1 is the temperature (°C) and X_2 is the pressure (bar).

Lower acid (Figure 4A) values of the concentrates are found below of 10°C regardless of the pressure used. The lowest solids retentions (higher rejections) (Figure 4B) were obtained at pressures and temperatures on the ranges of 1-2 bar and 5-10°C. The results can be justified in this conditions decrease the passage of solids to the permeate stream causing more retention.

Conductivity of 5.13 and 5.24mS.cm^{-1} were observed (Table 3) in the concentrates and permeates, respectively, and none of the variables studied had a significant effect (P>0.05) on the conductivity (Pareto chart not present). Thus, high conductivity observed in permeate is due to the amount of inorganic substances (salts), since they are completely permeable.

The high rejection of protein was 72.53% (Test 5 at 10ºC and 2 bar). By the Pareto chart (Figure not show) was not observed significant influence (P>0.05) of the variables, within the studied range. In the literature, there are few studies that used of flat membranes of 100 kDa in the whey processing. Although the used membrane of 100 kDa presents high MWCO, the results indicate that it may be a good alternative for pre-concentration of proteins (1.35g/100mL) at 3 bar and 15°C (Test 4).

For the retention of lactose, it was less than 5.5% (Table 3), however, not present significant effects (P>0.05) to temperature and pressure (Pareto chart not show). The obtained values indicate a partial retention of lactose which difficulty the proteins purification. Theoretically, a membrane of 100 kDa could not retain lactose, since this is a neutral solute and has less molecular mass than the cut-off molar mass of the membrane. However, fouling and the formation of a gel layer on the membrane surface could be factors that change the selectivity, resulting in rejection of some components with lower molar mass to cut off of the membrane.

A rejection of 22% of total minerals was obtained, but no present significative influence (P> 0.05) on pressure and temperature. The apparent rejection observed should be the layer formation on the membrane surface preventing the cross of these solutes. Based on the results obtained for the permeate flow of whey to the membrane of 100 kDa, in which a flow limit for the process was observed at a pressure of 2 bar and temperatures on the range of 5 to 10°C are more recommended were occur greater rejection of solids and protein. In this sense, were development new experiments using membrane of 50 and 10 kDa, 10°C and 2 bar.

Effects of pressure and temperature on the membrane of 50 and 10kDa

For the membrane of 50 and 10ºkDa, the conditions of 10°C and 2 bar were chosen, based on the results obtained for the permeate flow of whey

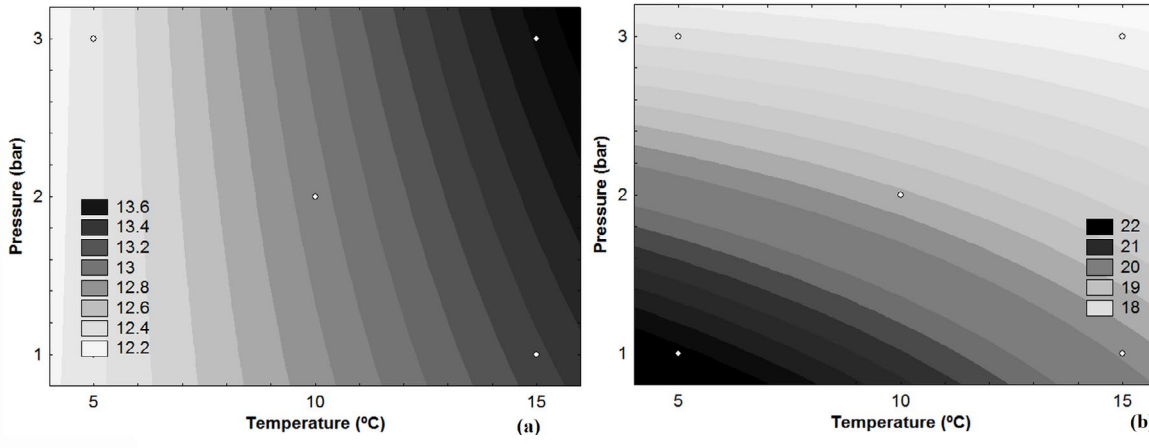

Figure 4 - Contour curves for acidity (a) and rejection coefficient for total solids (b) of concentrate whey obtained in UF process with a flat membrane of 100 kDa in the Design 2.

membrane of 100 kDa, which presented a limit flow (when an increase pressure no leads to a significant increase in flow). Also, 10°C seems to be more indicated, since the temperature of 15°C shows a tendency for small acidifications in the whey and temperatures of 5°C resulted in lower flow rates.

Was observed in the first 20min the permeate flow (10°C and 2 bar) a rapid and very sharp drop due mainly to the concentration polarization that occurs by increasing the solids concentration near the membrane surface. Over time (20 to 60min), a decrease flow occurred in smaller a dimension, which is caused by the particles deposition and retention under or inside the membrane pores, a phenomenon known as fouling. After 60min a slower decrease is observed, which may be due to a greater deposition of particles or consolidation of the fouling layer (Figure not shown). After 90min the permeate flow had a reduction of 50.80%, from 11.12L m^{-2} h^{-1} at the beginning of the process to 5.47L m^{-2} h^{-1}, again evidencing the phenomena of polarization by concentration and Fouling.

In relation to the mean flow rate, the membrane of 50 kDa presented a flow of 7.19L$m^{-2}h^{-1}$, which was 59.42% lower than that obtained for the membrane of 100 kDa under the same conditions (10°C and 2 bar). This reduction was expected since the membrane has a much lower pore size, in addition the membranes present differences in their composition which may alter the mode of interaction of the whey with the membrane material.

MACEDO et al. (2015), emphasize that in UF of whey the proteins (mainly β-lactoglobulin (β-Lg) and α-lactalbumin (α-La) and minerals, especially calcium and phosphate, are the main contributors to the fouling of membranes. In addition, other recognized contaminants components at long time that are processing residues such as rennet, residual lipids, enzymes and microorganisms are prone to adsorb on the surface of the membrane, or promote gelation in the polarized layer or further induce the blocking of the pore, making the fouling even more complex.

Table 4 shows the physicochemical characterization of *in nature* whey and of concentrate and permeate fractions, as well as the rejection coefficient obtained with membrane of 10kDa at 10°C and 2 bar. Protein values obtained for membrane of 10 kDa were shown to be higher for the concentrated fraction and lower for the permeate fraction when compared to the membrane of 50 and 100 kDa under the same UF conditions. In terms of concentration percentage in relation to the *in nature* whey, the membrane of 10 kDa presented concentration of approximately 44%. This value was about 3 times higher than obtained for the membrane of 50 kDa, which had a concentration percentage of 16%. The same behavior was observed for the solids content.

For the total minerals, conductivity and acidity, there was no significant difference ($P<0.05$) between *in nature* whey and concentrate and permeate fractions. Already for pH, the permeate and concentrate fractions showed a slight increase, differing statistically from each other ($P<0.05$).

The membrane of 10 kDa showed 80.48% of rejection for proteins, higher than obtained for the membrane of 50 kDa (71.43%) under the same process conditions, where this behavior also was verified for solids and lactose. The higher retention rates for the membrane of 10 kDa are attributed mainly to the smaller pore size, for lactose in specific, the retention observed is due to changes in the selectivity of the membrane caused due to the deposition of whey components that are deposited on the membrane, creating one additional resistance and hindering the permeation of lactose molecules.

To verify the main constituents that representing the proteins of concentrates and permeates obtained with the membrane of 10 kDa, an electrophoresis analysis was performed, and the figure 5 shows the

Table 4 - *In nature* whey constituents, concentrate and permeate fractions and rejection (%) obtained by UF with membrane of 10kDa at 10°C and 2 bar.

Constituents	*In nature*	Concentrate	Permeate	Rejection (%)
Protein (g 100 mL^{-1})	0.82^{b}±0.03	1.46^{a}±0.03	0.16^{c} ±0.02	80.48
Solids (g 100 mL^{-1})	5.81^{b}± 0.03	6.78^{a}± 0.02	4.15^{c} ± 0.09	28.57
Lactose (g 100 mL^{-1})	4.96^{a}± 0.01	4.41^{b}±0.08	4.16^{c}± 0.0	16.12
Minerals (g 100 mL^{-1})	0.57^{a}± 0.04	0.48^{b}±0.03	0.49^{b}±0.02	14.04
Conductivity (mS cm^{-1})	5.68^{a}± 0.04	5.61^{a}± 0.06	5.77^{a} ± 0.09	-
Acidity (°Dornic)	12.67^{a}± 0.57	13.33^{a}± 0.57	13.00^{a} ± 0.50	-
pH	6.51^{c}± 0.01	6.55^{b}± 0.01	6.58^{a}± 0.02	-

*mean (± standard deviations) followed by same letters on lines represents no significant difference at 5% level (Tukey´s test).

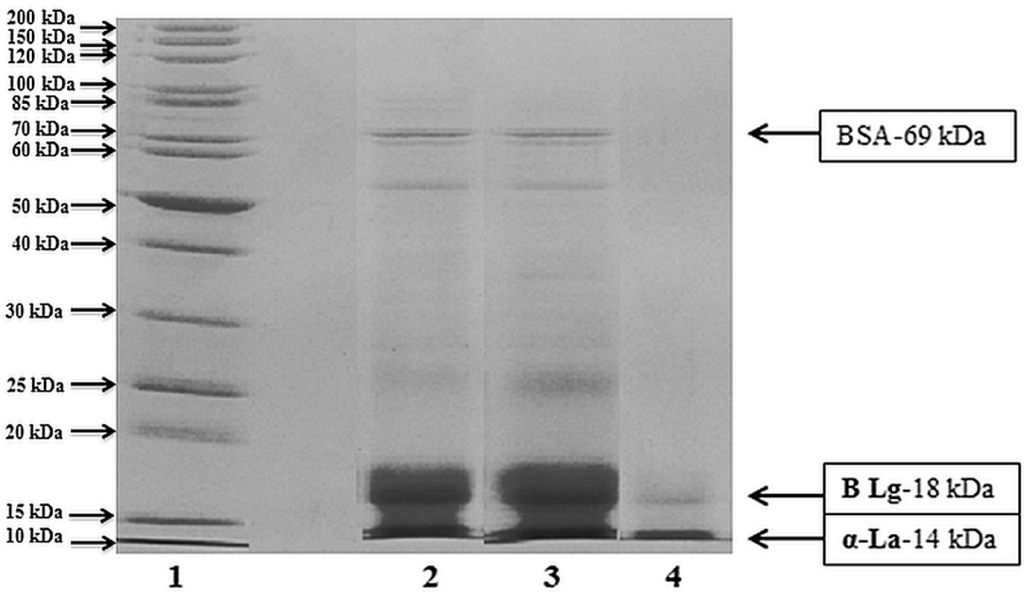

Figure 5 - Image of electrophoresis gels SDS-PAGE for UF permeate and concentrate samples of membrane of 10 kDa. From left to right: Column 1- molar mass marker; Column 2- *In nature* whey; Column 3- concentrated whey after UF; Column 4- permeated whey after UF.

image of the electrophoresis gel. It is observed in the electrophoresis gel that the concentrate sample shows a weak signal for both proteins, β-Lg (β-lactoglobulin of 18.3kDa) and α-La (α-lactalbumin of 14.2kDa), indicating that the two proteins are in the concentrate the membrane, since the membrane was 10kDa, thus retaining proteins of molecular weight greater than 10kDa. For the permeated fraction it is not noted a band BSA (69kDa).

Furthermore, the presence of other characteristic bands of minority proteins in the whey is verified, sub-fractions with different molecular weights, or even conformational variations of the proteins. These observations are in accordance with HARAGUCHI et al. (2006) which affirm that the fractions or whey peptides are composed of β-Lg, α –La, BWA Immunoglobulins (Ig's) and glycomacropeptides (GMP), as well as sub-fractions or secondary peptides that may vary in size and molecular weight.

Table 5 presents the results of the analysis carried out on the samples of *in nature* whey and the fractions (concentrate and permeate) of membrane of 50 and 10kDa. The constants K_h and K_s correspond to the size of the droplets generated, where K_s is related to large droplets and K_h with small droplets. As these

Table 5 - Values total protein, soluble protein, K_h and K_s for *in nature* whey, concentrate and permeate whey by membrane of 50 and 10 kDa.

Sample	Total protein (%)[1]	Soluble protein (%)[1]	K_h (min^{-1})	K_s (min^{-1})
In nature whey	12.39[b]	41.39[b]	$(6.7\pm2.0).10^{-4\ b}$	$(7.1\pm0.3).10^{-3a}$
Concentrate 50 kDa	12.69[b]	57.82[a]	$(1.1\pm0.1).10^{-3a}$	$(5.8\pm0.6).10^{-3b}$
Concentrate 10 kDa	22.66[a]	54.40[a]	$(7.2\pm0.1).10^{-8c}$	$(1.2\pm0.2).\ 10^{-3c}$

*means (± standard deviations) followed by same letters on lines/columns represents no significant difference at 5% level (Tukey´s test).
[1]Expressed in dry basis.

constants represent the destabilization of the emulsion, the larger the droplets the more unstable the emulsion will be. The concentrated fraction of 10kDa showed lower K_s, than, has more stable emulsions. The small drops represented by K_h are those that form cream more slowly with time, also corresponding to the concentrated fraction of 10kDa. According to MCCLEMENTS (1999), the droplet size produced during the formation of the emulsions depends on two processes: the generation of small droplets and the rapid stabilization of these droplets, which avoids their coalescence.

In summary, the viability of the whey protein concentration process by membranes depends, largely, of the conditions as the membrane properties, flow type, membrane-solute affinity, solution temperature, pressure, among others.

CONCLUSION

Considering that the present study was carried out in a laboratory scale with conventional flow and flat membrane of 100, 50 and 10kDa, the viability of the process will depend on a scale up, preferably employing a tangential flow and ceramic membrane, which would reduce operating times, cleaning steps and polarization phenomena by concentration and fouling.

Recovery of whey constituents by UF is of great interest to the industry, being an alternative of adding value to the subproduct of the dairy industry. The concentrated obtained by UF in laboratory scale showed a mean protein retention of 55 to 80% for membrane of 100 and 10kDa, respectively. Indicating the possibility of use in food industries with better emulsifying properties than the *in nature* whey.

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DECLARATION OF CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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