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Recovery of proteins from EDAM whey using membrane ultrafiltration

F. Yelles- Allam, A.A. Nouani

Laboratory of Food Technology, Faculty of Engineering Sciences, University of M'Hamed Bougara, 35000, Boumerdes, Algeria

*Corresponding author: allam.2006@yahoo.f

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ABSTRACT/RESUME

In Algeria, whey is discharged without any treatment and this causes not only pollution problem, but also a loss in nutritive components of milk. In this paper, characterization of EDAM whey is done, which is resulted from pasteurised mixture of cow's milk and skim milk. The recovery of whey protein by ultrafiltration/diafiltration, was studied. The physical-chemical analysis of whey has emphasized on its pollutant and nutritive characteristics. In fact, its BDO_5 and CDO are 49.33, and 127.71 g of O_2/l of whey respectively. It contains: fat (1,90±0,1 gr/l), lactose (47.32±0,57 gr/l), proteins (5,82±0,4gr/l) and ashes 4.53±0.15gr/l, calcium (0,518gr/l), Na (1.104gr/l), K (1.014 gr/l), Mg (0.118 gr/l) and P (0.482 gr/l). Tangential ultrafiltration of whey after treatment was carried out in a polyetersulfone membrane with a cut-off of 10K. Its hydraulic intrinsic resistance (R_m) and permeability (L_p) are respectively: $2.041.10^{12}$ m^{-1} and 176, 32 l/h.m^2bar. The dry matter obtained at FC6 is of 70.25 g/l, containing 16.33g/l of proteins. The retention rate of protein is 98.18 % and the decrease in BDO_5 and CDO are at 18.875 and 42.818 g of O_2/liter of permeate respectively. Diafiltration performed on concentred retentat allowed the complete removal of lactose and minerals. The ultrafiltration of the whey before the disposal is an alternative for Algéria dairy industry.

I. Introduction

Whey is a by-product from the cheese or casein manufacturing .This effluent is a greenish-yellow liquid and can be considered as milk free of casein and fat (Daufin et al., 1998) [1]. Whey contains more than half of the solids present in the original whole milk (A. Rektor;2004 [2]. It contains proteins (protein 5.36–7.42 g/l), lactose (35.90–44.26 g/l), fat (<2.5 g/L) and Ashes 3.77–5.39 g/L) (Eva Suárez and al 2006) [3].

However Cheese whey is considered the most important pollutant in dairy wastewaters, not only because of the high organic load, but also for the volume generated. The amount of whey produced is related to the productivity of cheese, According to Fox and al.; 2000 [4] producing 9,86 kg of cheese requires 100 kg of cow's milk and generates 0,873 liters of whey per liter of milk . In Algeria the totality of dairies in dispose of their waste, especially cheese whey, into the environment in enormous quantities. This causes serious pollution problems since whey has a high chemical oxygen demands (COD) (60–80 g/l) (Mockaitis et al.,2006) [5] and a high biochemical oxygen (BDO_5) 40 ± 2.55 (Gannoun and al ; 2008) [6] .

Whey proteins have a high nutritional value because of the high content of essential amino acids compared to other typical food proteins (Geoffrey, 2008) [7]. In addition to the nutritional properties, whey proteins have functional properties that confer beneficial functional properties when being used as

ingredients in food, mainly because of their good solubility capacity, water absorption, gelification and emulsifiers (Maubois, 2000)[8]. Due to the excellent nutritional and functional value, the commercial value of whey protein concentrate is 3-40 times higher than that of whey powder [9].
Membrane technologies can be used to firstly remedy the environmental pollution problem and secondly recover the added value whey components. For example, whey ultrafiltration is used for the recovery of proteins, and permeates nanofiltration for recovering lactose because lactose and proteins are major causes of pollution.
In this paper, characterization of EDAM whey, and recovery of whey protein by ultrafiltration/diafiltration was studied. Ultrafiltration is performed using a 10 kDa polyethersulfone membrane, at pH 6.3; transmembrane pressure of 1 bar, flow 40ml / minute and temperature of 30 °c.

II. Matériel et méthodes

Whey samples used in this study were supplied by a local dairy factory in Boudouaou (Algeria). It results from manufacture EDAM cheese. Samples of whey were taken in the coagulation tank, in sterile bottles and stored at 4°c until analysis and processing.

II.1. Treatment of whey

In order to enhance permeate flux; first, raw whey is filtered to remove suspended particles (fine casein). Then Clarification was performed by thermal aggregation of phospholipoprotein calcium complex. $CaCl_2$ was added to raw whey at 5-8 °c to get a calcium concentration of 1.2 g / l. pH was raised to 7, 3 using 35% NaOH and temperature were increased to 55 °c. These conditions were held for 5 minutes, involving the aggregation of complex lipid-calcium phospholipoproteines particles. Finally, whey was cooled down to 10°c. The precipitate formed is separated by decantation and centrifugation with HERMLE centrifuge type Z 36 HK at 4000rpm for 30 minutes, and the supernatant was recovered. Clarified and raw whey were analyzed for proteins, lactose, lipids, ash, dry extract , mineral (Ca, P, K, Na, Mg) , BDO_5 and DCO.

II.2. Ultrafiltration experiments

Experiences runs were performed using a minimate tangential flow filtration TFF capsule OA010C12 (Ann Arbor USA) of dimensions 20 cm x 3.8cm x 1.8 cm (LxWxH). Effective area of membrane was 50 cm^2 with molecular mass cut-off of 10. Whey circulates from a reservoir of 500 ml containing 300 ml of product, using a peristaltic pump FS700M01. Two pressure gauges FS700X14 type are used, one at the inlet and the other at the outlet of the membrane to measure the transmembrane pressure (TMP). Liquid is homogenized using a magnetic plate.
Permeate flux (J_W) of distilled water is determined volumetrically at 20 °c and at transmembrane pressure ranging from 0.35 to 1.35 bars. The valve on the retentate side is fully tightened.
Flux permeability (Jw) is calculated from the time of collecting 20 ml of permeate with equation [1] :

$$J_w = \frac{1}{A_m} \times \frac{\Delta V}{\Delta T} \quad [1]$$

Am: membrane area. Δv/ Δt (l / h): collected volume of filtrate on time.

This test allows plotting water flux (Jw) according to PTM. A clean membrane has a pure water flux (Jw) which may be expressed according to equation [2]

$$J_w = \frac{PTM}{\mu R_m} = L_p \, PTM \quad [2]$$

μ: viscosity of distilled water = 10^{-3}Pa.s ,TMP: transmembrane pressure = 1 bar , L_p : membrane permeability L/hm^2bar and Rm: Intrinsic resistances of a clean membrane and serves as reference in order to verify the effectiveness of the chemical cleaning of the membrane after use. Ultrafiltration/diafiltration of clarified whey is carried out in the following operating conditions: PTM of 1bar, Flow rate of 40ml/min and pH of 6.3. The pH value of 6.3 was selected because that is the optimum pH obtained previously in another study **Yelles et al 2015** [10] using a PES membrane of 30 kDa cut-off. During circulation of whey, permeate is collected in a graduated cylinder, while retentate is returned to the tank. The flux decline was determined volumetrically at each concentration factor (CF). Immediately after concentrating clarified whey to FC6, a diafiltration with 4 diavolumes of distilled water was performed to remove lactose and minerals, without membrane cleaning between the stage of ultrafiltration and diafiltration. Diafiltration was used to purify proteins concentrated by adding a volume of distilled water equal to that of concentrated whey. Retentate was then concentrated to FC 10. Samples of permeate (in graduated cylinder) and retentate (in tank) were collected after each concentration factor (CF) of 1.5; 2; 3 and 6 and after each diavolume for determining total solid and proteins. This experiment was performed in triplicate, with the same membrane after chemical cleaning. The concentration factor was determined as the relationship between initial and final volume in the feed tank.

The retention rate is determined for concentration factors of 1.5, 2, 3, and 6 and expressed using the following equation:

$$TR = 1 - (Cp/Cr) \times 100 \quad \textbf{(4)}$$

Where Cp is protein concentration in permeate and Cr is protein concentration in retentate.

Protein yield was calculated, at FC10, using the following equation:

$$Y = Vr \times Cr / Vf \times Cf \quad (5)$$

Y: protein yield (%)
Vr: volume of concentrated retentate (L)
Cr: protein concentration in the volume Vr (g/L)
Vf: feed volume (L)
Cf: protein concentration in the feed (g/L).

II.3.Analysis method

The following parameters were analysed:pH using a Jenway 3510 pHmeter, dry matter by drying for 4 hours at 105°C in a Heaeus electronicoven, fat by the Gerber method **[11] NF04-210**, in a FunkeGerbercentrifuge, and ash by incineration at temperatures between 530-600°C**[11] NF04-208,** in a Nüve hang MF 120 muffle type-furnace for 6 hours. Protein contents of the raw whey, feed (clarified whey), retentate and permeate were determined according to the Folin-Lowry method at 650nm using bovine serum albumin (BSA) as the reference **[12**]. Lactose was quantified by the Bertrand method **[13],** the estimation of phosphorus was performed by spectrophotometry at 820 nm [10] on a UV/VIS type Unicam UV/VIS spectrometer, calcium, potassium, Phosphorus, magnesium and sodium was measured by atomic absorption spectrometry on an FS 95 Furnace auto-sampler. Chemical oxygen demand (DCO) was determinate using the method described by **Franck Rejsek .P (2002**) **[14**]. Biochemical oxygen demand (DBO_5) was measured using the method described by **Rodier.J & al (1996**) **[15**], in a thermostated enclosure WTW OXITOP BOX, at 20±2°c. All determinations were made in triplicate

III. Results and discussion

III.1. Composition of raw and clarified whey

Analysis of raw whey composition (Table 1) reveals two characteristics of particular importance. First, whey essentially contains 47.32 g / l of lactose, and 5.82 g / of proteins. These components are responsible for the high putrescibility and biological demand for oxygen (BDO_5) of whey (Geoffrey.W.S; 1996) [16]. It was observed that the chemical oxygen demand (DCO) and the biochemical oxygen demand (DBO_5) are very high. They are of 49.33 and 127.71grams of O_2 per litter of raw whey respectively. The clarification treatment, applied in order to increase the permeation flux during ultrafiltration results in the total elimination of the residual fat(Table 1) which is responsible for membrane fouling, and a reduction in protein by 16.32**%.** Fauquant et al 1985 by applying a heat treatment at 79 ° C for 8 seconds have obtained 17% loss in protein. These results also showed a decrease in minerals content.

***Tableau 1**: Composition of raw and clarified whey*

	Raw whey	Clarified whey
BDO g of O_2/l of whey	49.33	-
CDO g of O_2/l of whey	127.71	-
pH	6,3 ±0,01	7.30 ±0.01
Total solid	58.9± 0.65	53.64±0.6
Fat (g/l)(g/l)	1.90±0.1	0.00
Lactose (g/l)	47.32±0.57	44.8 ±0.85
Proteins (gr/l)	5.82±0.4	4.87±0.2
Ash (gr/l)	4.53±0.15	3.98±0.41
Phosphorus (gr/l)	0.482	0.188
potassium (gr/l)	1.014	0.99
sodium (gr/l)	1.104	1.05
calcium (gr/l)	0.518	0.459
Magnesium (gr/l)	0.118	0.109

III.2. Membrane characterization (resistance (Rm) and permeability)

Fig.1 shows the water-permeate flux (J_W) as function of transmembrane pressure and indicates that flux of water increased linearly with transmembrane pressure (R^2=0.998).The series of water run was taken for evaluate membrane hydraulic resistance Rm and permeability (Lp) which are found to be $2{,}04.10^{12}$ m^{-1} 176.32 l/m^2h bar respectively. Darcy's law (equation 2) was used to estimate Rm.

III.3. Characterization of permeate and retentate ultrafiltration / diafiltration

The results obtained during the concentration of whey are shown in figure 2. Permeate flux (J) is plotted vs. VCR. It can be observed that flux decreases with increasing concentration, as expected, mainly due to the increase in the osmotic pressure and polarization de concentration. The perméate flux (J) the volume of liquid removed per unit membrane surface per hour (Lo et al., 1997)

[17], declined rapidly from 36 ±1,227 to 28.11± 1,243 l/hm^2 at the concentration factor of 1.5 and continued to drop slowly until the end of the process to reach 25.35± 1,293 at CF 6. Similar flux profiles are obtained by Su-Hsia Lin and al; 2008[18]; C. Baldasso and al; 2011[19].

Fig 1*: Water-permeate flux as function of transmembrane pressure at 20°c.*

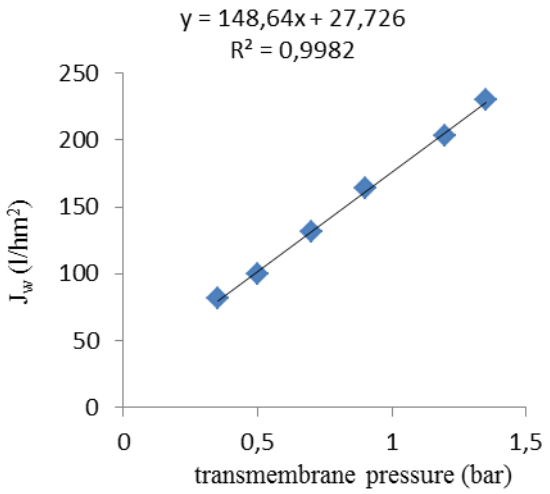


Fig 2: *Flux Permeation of whey vs. CF at pH: 6.3, flow rate:40 ml / min, T°: 30°C and TMP:1 bar)*

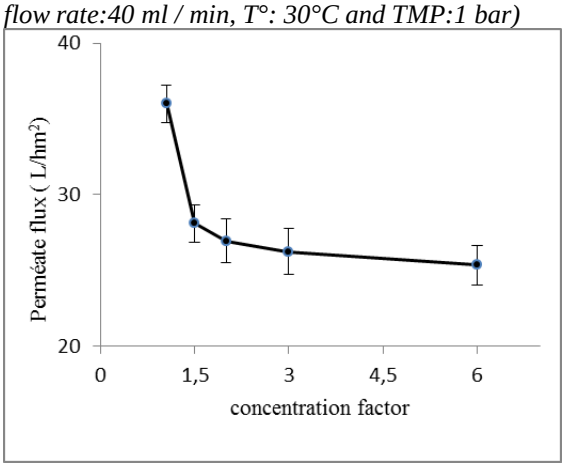
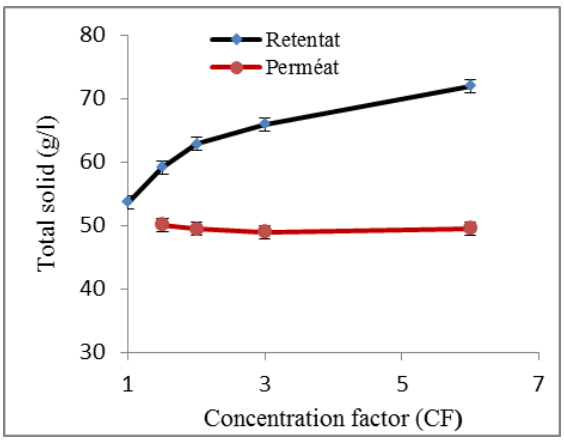
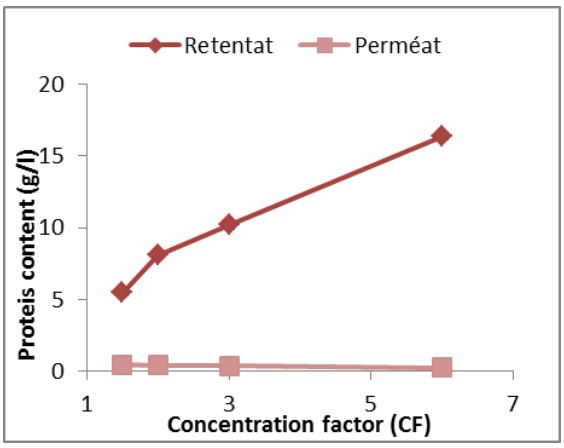


Figure 3 shows variation of total solid (a) and proteins (b) between permeate and retentate during ultrafiltration vs CF (1.5; 2 ; 3 and 6) at pH 6.3; flow rate: 40 ml/mn; PTM: 1bar and T°30°c, below the protein denaturing temperature. In retentate, total solid and proteins increases with increasing the concentration factor. (Fig 3a) shows that concentration of total solid increases from 53.64 g / l, at concentration factor of 1(CF1), to 71.92 g/l, at concentration factor of 6 (CF6). However, in permeate, the concentration of total solids is constant for all CF, it is of 50.09, 49.45, 48.93 and 49.52 g/l for the CF respective of 1.5, 2, 3 and 6. also the solids in the permeate is high, and this is due to the transmission of the components of which molecular weight is significantly lower than the membrane cut-off, particularly lactose, which is the predominant component of the whey (see Table 1). Figure (3.b) shows that proteins content increased from 4.87 at CF1 (see table 1) to 16.33 at CF6, yielding a concentration factor of 3.35.This concentration factor, lower than the theoretical value six (CF6) determined by the change in liquid volume after UF, indicates loss of proteins that fouled membrane. In permeate, the amount of protein is low, and it is of 0.453±0.025, 0,436±0.015, 0.4±0.02 and 0.283±0.028 for the CF respective of 1.5, 2, 3 and 6. The evaluation of protein retention rate with the formula (4) shown in Figure 4 indicates that the rate of protein retention increases with the concentration factor, it increases from 91.74 ± 0.0.512 at CF1.5 to 98.18 ± 0.32 at CF6.

Figure 3: Variation of total solid (a) and proteins content (b) between permeate and retentate during ultrafiltration vs CF (1.5; 2 ; 3 and 6) at pH 6.3; flow rate: 40 ml/mn; PTM: 1bar and T°30°C.



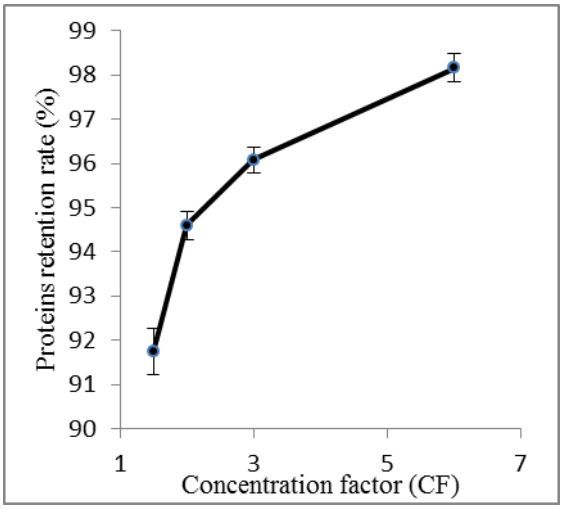
(a)



(b)

The majority of whey proteins are retained by the membrane of 10 kDa. The most abundant whey proteins are β- lactoglobulin 3.2 g / l (Eugenia et al.2006) [20] and α-lactalbumin1, 2 g/l(Etezel , 2004) [21]. The molecular weight of these two proteins are 14 KDa for α-lactalbumin and 18 KDa for β- lactoglobulin (Etezel , 2004) [21] which are higher than that of the membrane. In addition β-lactoglobulin exists in dimeric form (36,7KDa) at pH above its isoelectric pH (5.2).

***Figure 4**: Retention rate of the protein vs. Concentration factor*

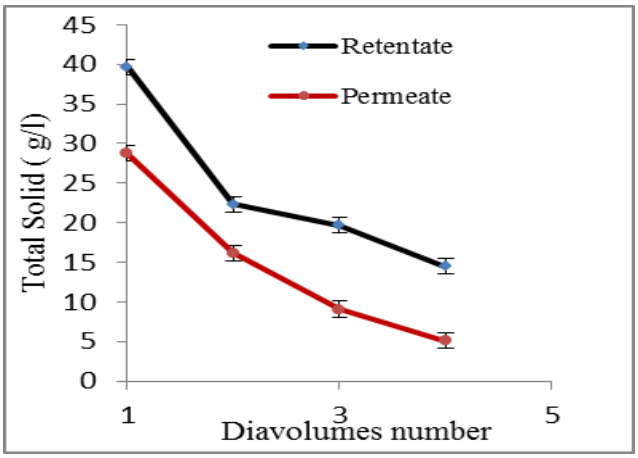


Variation of total solid (a) and proteins (b) between permeates and retentate during diafiltration vs. number of diavolumes at pH 6.3; flow rate: 40 ml/mn; PTM: 1bar and T°30°C are shown in figure 5.

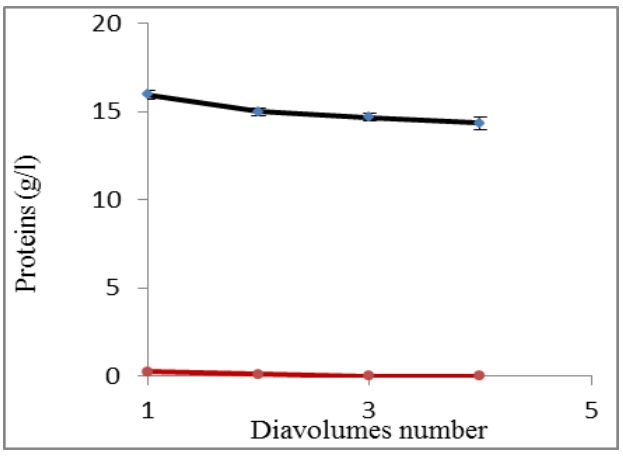
The results show that diafiltration leads to a gradual decrease in the amount of total solids after each diavolume (step of diafiltration) . In the retentate amount of solid gradually decreases from71.92 g/l (total solid in CF6) to 39; 66; 22.32; 19.69 and 14.49 g/l respectively after the first, second, third and fourth diavolume of distilled water.

This result was caused by the gradual elimination of lactose and salts which have passed through the membrane. The rate of protein concentrates at CF6 decreased slightly during the diafiltration, it dropped from 16,33 g/l in CF6 to 14,33 g/l in retentate after the fourth diavolume of diafiltration. This decrease in proteins is caused mainly through their participation in membrane fouling. Indeed, the transmission of protein in permeate is very low (0.2g / l) while the loss between CF6 and fourth diavolume diafiltration is 2 g /l. After processing, a significant portion of the product is on the membrane in the form of a "gel layer" and needs to be recovered back into the solution before the system is drained. Recirculating 10 ml of distilled water can recover most of this gel layer. The yield is determined, at CF10 (30ml of concentrate) added with 10 ml of protein solution which was on the membrane. The proteins yield is calculated with the formula (5) and it was of 79, 39%. Analytical results of BOD_5 and COD in permeate samples showed that there is a decrease of these two parameters. BOD_5 and COD decreased respectively from 49.33 and 127.71 gram d'O_2/ liter of raw whey to 18.875 and 42. 818 gram d'O_2/ liter of permeate whey. Therefore ultrafiltration is an effective technique that can be used in dairy industries for the treatment of wastewater and thus fight against the environmental pollution.

***Figure 5**: Variation of total solid (a) and proteins (b) between permeates and retentate during diafiltration vs. number of diavolumes at pH 6.3; flow rate: 40 ml/mn; PTM: 1bar and T°30°C.*



(a)



(b)

IV. Conclusion

The physicochemical characterization of EDAM whey showed its high content in lactose and proteins that are responsible of the higher BDO_5 and CDO. Ultrafiltration of whey after clarification has allowed firstly to concentrate and recover proteins, and secondly to reduce the BDO_5 and CDO from 49.33 and 127.71 gr of 0_2/l of raw whey to 18.875 and 42. 818 gr of 0_2/l of permeate d' ultrafiltration. This decrease is important, but it remains high. Therefore, for obtaining zero pollution, it is necessary to recover lactose for example by nanofiltration.

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