Whey Ultrafiltration: Effect of pH on Permeate Flux and Proteins Retention

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Abstract: Ultrafiltration is an interesting technique that is widely used to valorize whey proteins. However, it is limited by membrane fouling. Whey clarification and the operation in appropriate conditions attenuate this undesirable phenomenon. In order to improve ultrafiltration efficiency, a clarification processing was performed followed by ultrafiltration and diafiltration. The chemical composition of clarified whey and UF/DF retentate and permeate was studied. The tangential ultrafiltration of clarified camembert whey was realized with a 30 kDa polyethersulfone membrane. Its hydraulic intrinsic resistance and permeability were 1,104 10^{-3} m^{-1} and 326, 3 L/h/m^2, respectively, at 1 bar PTM. Whey was adjusted to pH 5, 6.2 and 7.3 and ultrafiltration was carried out to elicit the effect of pH on permeate fluxes and protein retention. At pH 6.2 and 7.3, permeate fluxes ranged from 50.40 to 39.36 L/h/m^2 and from 55.2 to 33.96 L/h/m^2, respectively. On the other hand, around the isoelectric point of major proteins, at pH 5, permeate fluxes varied from 38.4 L/m^2 h to 25.58 L/m^2 h. For protein retention at CF 3, the best result of 96.2% was obtained at pH 5. Diafiltration performed on protein concentrates allowed the complete removal of lactose and minerals.

Key words: Camembert whey • Clarification • Ultrafiltration • Diafiltration • Proteins retention • Permeate flux

INTRODUCTION

Whey is the by-product of cheese manufacture, it is rich in lactose and proteins and is a redoubtable pollution factor when is not treated. Proteins are the major attraction of whey as a result of the opportunities available in the food industry. Indeed, it is used as an ingredient in various foods, not only because of its nutritional value, but also because of its advantageous properties: emulsifying, gelling and foaming [1,2,3]. For recovering the protein fraction, membrane separation techniques have opened up an important economic perspective in some countries and this process allows pollution and valorization to be coupled. They are widely used in the dairy industry for the standardization of milk protein, as well as for the recovery of whey proteins [2]. Whey protein concentrates (WPC) contain 50-75% of protein [4,5]. However in ultra-filtration, two main phenomena reduce productivity. These are concentration polarization and fouling of the membrane, which decreases the permeate flux and modifies the membrane selectivity. Both phenomena depend on the membrane (cutoff, materials), operating conditions (pH, temperature, solution concentration) and hydrodynamic conditions (tangential velocity, transmembrane pressure). The main components that can cause fouling in ultrafiltration of whey are residual fat, casein fines, calcium phosphate and proteins. Several methods have been proposed to enhance membrane performance and reduce membrane fouling such as pretreatment of feed [6,7], installation of turbulence promoters [8,9], modification of membrane surface [10,11] and proper selection of operating conditions in particular the feed pH.

In Algeria, pollution caused by whey is an underestimated if not totally ignored threat. The quantity of whey rejected into the environment is constantly...
increasing. If we consider only the dairy industry of
Draa Ben Khadda (Algeria), whey produced each year, by
the manufacture of camembert cheese, was estimated to be
5,840,000 liters, which is equivalent to 581,605 kg of
nutritive elements that are purely and simply considered
effluents.

Our work aims to recover camembert whey proteins
by tangential ultrafiltration followed by diafiltration and to
demonstrate the effect of pH on the permeation flux and
the retention rate of protein. A polyethersulfone (PES)
membrane Omega with a 30 kDa molecular weight cut-off
(MWCO) was used for this study.

**MATERIALS AND METHODS**

Whey samples used in this study were supplied by a
local dairy factory in Draa Ben Khedda (Algeria). These were
the result of camembert cheese manufactured from cow's milk. The samples of whey were taken into the
coagulation tank, in sterile bottles and stored at 4°C until
analysis and processing was performed.

**Whey Treatment**: In order to enhance permeate flux,
wey was clarified. This treatment was performed by
thermal aggregation of the phospholipoprotein-calcium
complex, as described by Fauquant and al [12] and
modified by Pereira and al, Almécia and al [13, 7].
First, CaCl₂ (0.8 g/L) was added to whey at 5°C. Then, the
pH was raised to 7.3 using 35% NaOH and the temperature
was increased to 50°C. These conditions were
held for 8 minutes, enabling the aggregation of complex
lipid-calcium phospholipoprotein particles. Finally, whey
was cooled down to 10°C. The precipitate formed was
separated by decantation and centrifugation with a
HERMLE centrifuge type Z36 HK at 4000 rpm for 30 min.
The supernatant (clarified whey) was recovered and
characterized.

**Ultrafiltration of Clarified Whey**: Experiment runs were
performed using minimate tangential flow filtration (TFF)
capsule OA010C12 (Ann Arbor USA) with the dimensions
20 cm x 3.8 cm x 1.8 cm (LxWxH). The PES membrane was
encased in a polypropylene housing with connectors on
the feed, retentate and filtrate for easy connection to a
pump and accessories. The effective area of the Omega
membrane was 50 cm² and the molecular weight cut-off
(MWCO) was 30 kDa.

The clarified whey circulates from a reservoir of
500 ml containing 300 ml of product, using a peristaltic
pump FS700M01. Two type FS700X14 pressure gauges
were used, one at the inlet and the other at the outlet of
the membrane, to measure the transmembrane pressure
(TMP). Liquid was homogenized using a magnetic plate.
During the circulation of whey, the permeate stream of
each experiment was steadily removed and collected
separately in a graduated cylinder, while the retentate
stream was recycled to the feed reservoir in order to
ensure the maximum solute concentrate.

**Determination of Permeability and Rm of Membrane**: Water
permeability of a membrane depends on the hydraulic resistance (Rm) of the clean membrane at a fixed
transmembrane pressure. The flow permeability of distilled
water was determined volumetrically at 20°C and at a
transmembrane pressure ranging from 0.45 to 1.55 bars.
The valve on the retentate side was fully tightened. Flow
permeability (Jw) was calculated from the time taken to
collect 20 ml of permeates using equation (1):

\[ J_w = \frac{1}{A_m} \frac{\Delta v}{\Delta t} \]  

- Am: membrane area
- \( \Delta v/\Delta t \) (L/h): collected volume of filtrate on time.

This test allowed us to plot the permeate flux of water
(Jw), according to PTM, to calculate the permeability of
the membrane at a TMP of 1 bar, in order to determine
hydraulic resistance (Rm) of the Clean membrane and
determine the effectiveness of chemical cleaning of the
membrane after use.

Chemical cleaning of the membrane was achieved
using a solution 0.5 N NaOH at 45°C for 60 min followed
by rinsing with distilled water at 45°C for 30 min.

The intrinsic resistance of the membrane
was determined using Darcy's law (equation 2):

\[ J_w = \frac{PTM}{\mu Rm} \]  

- \( \mu \): viscosity of distilled water = 10⁻³ Pa.s
- TMP: transmembrane pressure = 1 bar
- Rm: intrinsic resistance of the membrane.

The effectiveness of a cleaning protocol of the
membrane after use was evaluated by the water
permeability of the membrane after chemical cleaning with
that of a new membrane in the form below:

\[ Tr (%) = \frac{NWP \text{ (after cleaning)}}{NWP \text{ (new membrane)}} \]  

- Tr: the rate of membrane regeneration
Effect of pH on Ultrafiltration: Experiments of the influence of pH on flux permeation and protein retention were achieved by studying three pH values: 6.2, 7.3 and 5; these were adjusted by adding sodium hydroxide or chloric acid. These experiments were performed under the following operating conditions: Temperature: 30°C, retentate flow: 35 mL/min and PTM 1 bar.

The flux decline was determined volumetrically as Jw. During ultrafiltration, 10 ml of samples were taken in the retentate (reservoir) and permeate (graduated cylinder) at factors concentration (FC) 1.5, 2 and 3 for the determination of proteins, total solids, lactose and ash. The retention rate of proteins at different pH 5, 6.2 and 7 was determined at FC3 and expressed using the following equation:

\[ TR = 1 - \left( \frac{C_p}{C_r} \right) \times 100 \]  

- \( C_p \): protein concentration in the permeate
- \( C_r \): protein concentration in the retentate.

After concentrating the clarified whey to FC3, diafiltration with 4 diavolumes of distilled water was performed to remove lactose and minerals. Diafiltration was used to purify proteins concentrated by adding a volume of distilled water equal to that of concentrated whey. Retentate wash containing FC 5 and samples of permeate and diafiltration retentate were collected after each diavolume for the determination of total solid, proteins, lactose and ash in permeate and the determination of total solid and proteins in retentate. Protein yield was calculated, at FC 5, using the following equation:

\[ Y = \frac{V_r \times C_r}{V_f \times C_f} \times 100 \]  

- \( Y \): protein yield (%)
- \( V_r \): volume of concentrated retentate (L)
- \( C_r \): protein concentration in the volume \( V_r \) (g/L)
- \( V_f \): Feed Volume (L)
- \( C_f \): Protein Concentration in the Feed (g/L).

Analysis Method: The physicochemical analysis focused on the determination of pH using a Jenway 3510 pHmeter, dry matter by drying for 4 hours at 105°C in a Haeaeus electronicoven, fat by the Gerber method [14] NF04-210, in a FunkeGerbercentrifuge and ash by incineration at temperatures between 530-600°C[Afnor NF04-208, 1986] 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 [15]. Lactose was quantified by the Bertrand method [16], the estimation of phosphorus was performed by spectrophotometry at 820 nm [14] on a UV/VIS type Unicam UV/VIS spectrometer, calcium (Ca++) was measured by atomic absorption spectrometry on an FS 95 Furnace autosampler. All determinations were made in triplicate.

RESULTS AND DISCUSSION

Composition of Raw Whey and Clarified Whey: The composition of raw whey and clarified whey are shown in Table 1. This treatment leads to the total elimination of residual fat, which is responsible for the fouling of membrane ultrafiltration.

Clarification of whey is conducted to minimize membrane fouling caused by residual fat, lipoproteins and calcium and also to increase permeation flux. This treatment resulted in the elimination of fouling agent and has been proposed as an effective strategy for the enhancement of permeates flux [17, 6, 18, 7].

Clarification of acidic whey, proposed by Alméjija and al [7], significantly enhanced the permeate flux of whey when cross-flow ultra-filtered through a 50 kDa tubular ceramic membrane; flux was improved by a factor of 3-3.5 at a transmembrane pressure range of up to 2 bars.

Membrane Characterization: As shown in the curve in Figure 1, the permeation flux of distilled water increases in a linear manner with increasing transmembrane pressure. Flux at TMP 1 bar was 326.3 L/hm²; the intrinsic resistance of the clean membrane, evaluated by Darcy's law, was 1,104 \( 10^4 \) m⁻¹. After each use, the membrane was cleaned. The regeneration rate (TR) of the membrane was calculated using equation [3]. TR was 97.70%±1%. This result is above the limit value given by the manufacturer (75%). Therefore, we can conclude that chemical cleaning with 0.5 N NaOH is effective and membrane can be reused.

Effect of pH on Flux Permeation: Figure 2 shows the effect of pH on permeates flux at PTM 1bar, temperature 30°C and flow rate 35ml/min. This effect was observed at different values: around the isoelectric point of the most abundant whey protein (pH 5), the isoelectric pH of the β-lactoglobulin, α-lactalbumin and bovine serum albumin were 5.4, 4.4 and 5.1, respectively [19], at the raw whey pH (6.2) and at pH of the clarified whey (7.3). The permeate flux values declined mildly over time for the pH 5.0, 6.2 and 7.3, respectively.
The curves have a hyperbolic shape. We note that there are 2 phases: A rapid phase, in which flux decreases rapidly during the first twenty minutes, which is due to the formation of a concentrated polarization layer and a slow decline phase. This decline in the permeate flux is due to the gel layer formation on the membrane surface as the feed concentration increased and deposition occurred. These results are in agreement with the theory that has been previously outlined [20, 21, 22, 23, 24].

The graph shows us that during the first five minutes of ultrafiltration, the permeation flux was 55.2 L/m²h for pH 7.3, 50.4 L/m²h at pH 6.22 and 38.4 L/m²h for pH 5. The declination of the flux after 10 minutes of filtration, compared to the initial flux, was greater at pH 5, with values of 12.5%, 9.1% and 4.76%, respectively, for pH 5, 7.3 and 6.2. These results show that the polarization layer and fouling of the membrane were formed more rapidly at pH 5. Flux values obtained after about 60 minutes of filtration were 33.96, 39.36 and 25.58 L/m²h for the respective pH 7.3, 6.2 and 5. Filtration time to reach the concentration factor of 3 (FC3) was 60, 70 and 105 minutes for the respective pH 7.3, 6.2 and 5. Filtration time to reach the concentration factor of 3 (FC3) was 60, 70 and 105 minutes for the respective pH 6.2, 7.3 and 5.0. The lowest Flux permeation was obtained at pH 5, which was due to the development of membrane fouling caused by the deposition of aggregates of uncharged protein molecules which involved pronounced flux drop [25].

Sarkaret and al [23] when studying the effect of two pH values: 2.8 (away from the isoelectric point) and pH 5.5 (at about the isoelectric point), found a higher permeation...
The permeate flux of protein solution was lower at the isoelectric pH of the protein. For CF 3, the level of total solids was 6.7%, 6.52% and 7.2% for pH 6.2, 7.3 and 5, respectively. In contrast, in the permeate, the total solids were almost constant, whatever the value of pH and CF. With regard to concentration at CF 3, the level of total solids is of 4.92, 4.91, 4.79 % at pH 6.2, 7.3 and 5, respectively. The lowest value of the total solids in permeates and the highest value in the retentate was obtained at pH 5, probably because of the higher protein retention at this pH.

This figure reveals that the rate of proteins retained in the retentate increased with increasing concentration factor for the three values of pH studied. At FC 3, the rate of protein was 1.55%, 1.48 and 1.45% at pH 6.2, 7.3 and 5, respectively. The lowest value was obtained at pH 5,
Fig. 4: Variation of whey components between permeate and retentate during diafiltration vs. number of diavolumes (flow rate: 35 ml/min; PTM: 1 bar and T° 30°C). (a) total solid in retentate, (b) proteins in retentate, (c) total solid in permeate, (d) proteins in permeate, (e) ash in permeate, (f) lactose in permeate

because the amount of proteins that participated in the fouling of the membrane was greater at this pH. At the same time, in permeate the level of protein was constant with increasing concentration factors, whatever the pH value. At pH 5, the transmission of protein (0.055%) in permeate was slightly lower than at pH 6.2 (0.076%) and at pH 7.3 (0.07%). The 30 kDa membrane promoted the transmission of a significant amount of proteins. Several authors have noted this phenomenon using the same membrane cut-off.

Lactose and ash pass completely in ultrafiltration permeate, whatever the value of concentration factor and pH. The rate of permeation of lactose at CF 3 was 4.23%, 4.25% and 4.1% for the pH 6.2, 7.3 and 5, respectively. The ashes represent 0.305%, 0.29% and 0.28 at pH 6.2, 7.3 and 5, respectively.

Characterization of Permeate and Retentate of Diafiltration: Significant improvements in the efficiency of separation by ultrafiltration were accomplished by introducing a diafiltration step at a concentration factor (CF) of 3. Ultrafiltration retentate was diafiltered with four diavolumes of distilled water. The results shown in Figure 4 show the distribution of whey components between permeate and retentate during diafiltration. The rate of total solids of the retentate decreased from 4.09% to 1.45% at pH 6.2; from 3.55% to 1.29% at pH 7.3 and from 4.77% to 1.59% at pH 5 after the fourth diavolumes. This result was due to the decrease in the concentration of ash and lactose. Indeed, diafiltration led to the total elimination of ash and lactose content in the retentate.

The rate of protein concentrates at CF3 decreased slightly during the diafiltration, whatever the solution pH. It dropped from 1.47% to 1.34% at pH 6.2, from 1.32% to 1.02% at pH 7.3 and from 1.24% to 1.14% at pH 5. This decrease was mainly due to the transmission of proteins in the permeate. Indeed, the amount of protein permeate was 0.055% at pH 6.2, 0.05% at pH 7.3 and 0.04% at pH 5.
Table 2: Retention rate and yield of protein function of pH

<table>
<thead>
<tr>
<th>Parameters</th>
<th>pH: 6.2</th>
<th>pH: 7.3</th>
<th>pH: 5</th>
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<tbody>
<tr>
<td>FC: 3</td>
<td>P</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Proteins(%)</td>
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<td>1.55</td>
<td>0.07</td>
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<td>Retention rate(%)</td>
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<td>95.27</td>
<td>96.21</td>
</tr>
<tr>
<td>FC: 5</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Proteins (g/l)</td>
<td>20.2</td>
<td>16.48</td>
<td>16.9</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>62.69</td>
<td>51.14</td>
<td>52.45</td>
</tr>
</tbody>
</table>

Effect of pH on Protein Retention: The retention rate is determined at the concentration factor of 3 and the yield is calculated after the step of diafiltration and concentration to the concentration factor of 5.

According to Table 2, we find that the highest retention rate of proteins is obtained at pH 5 and is 96.2%, however the higher yield was obtained at pH 6.2 and is 62.69%. The low yields for the different pH studied are essentially caused by the transmission of a significant proportion of protein in permeate and also by protein participation to membrane fouling. Indeed, several authors have been able to quantify the proteins responsible for membrane fouling.

CONCLUSION

Ultrafiltration is a fascinating technique used to valorize whey proteins. The study of the effect of pH on the permeation flux with time for clarified whey showed that the declination of the flux after ten minutes of ultrafiltration compared to the initial flux was greater at pH 5. These results show that the polarization layer and membrane fouling are formed more rapidly at pH 5. Thus, if we take into account permeation flux with time as a criterion for choosing the optimal value of pH, it is clear that pH 6.2 provides the best results.

It was observed that pH solution had a strong effect on UF and therefore on the duration of ultrafiltration. The duration of ultrafiltration at pH 5 is 1.75 times higher than that obtained at pH 6.2. The low yields for the different studied pH values are essentially caused by the transmission of a significant proportion of protein in permeate and also by protein participation in membrane fouling. In order to improve performance, work with an ultrafiltration membrane weight cut-off of 10 kDa is being implemented in the laboratory.

REFERENCES