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Characterization of Milk Proteins in Ultrafiltration Permeate and Their Rejection Coefficients

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ABSTRACT: The most widespread method applied for standardization of milk protein in cheese production is ultrafiltration. In the current study, pasteurized, unhomogenized whole milk was ultrafiltered with APV ultrafiltration system, 51 spiral wound, polysulfone membrane with 20000 Da molecular weight cut off (DDS, Nakskov, Denmark) at 50 ˚C, such that the pressure difference between the two sides of membranes was not more than 3.6 bar. Permeate that was collected, contained (w/w٪) 0.01 ٪ proteins. The protein was isolated from crystalline TCA to the concentration of 12.5٪ (w/v), and then characterized by discontinuous SDS-PAGE (10-20 ٪ acryleamide). The products of proteolysis contained α-lactalbumin (49.95±2.25 %), β- lactoglubolin (22.30±2.3 %) and casein proteolysis products (30±4 %) and (96.68±1.725), (99.18±0.46) and (98.39±1.005) % were rejected by ultrafiltration membrane, respectively. Casein micelles were rejected completely (100 ٪), therefore, they couldn't penetrate to permeate from retentate. Incomplete rejection of casein and penetration of casein into permeate indicates the leakage of retentate in to permeate.

Keywords: *Milk, Permeate, Protein, Rejection Coefficient, Retentate, Ultrafiltration*.

Introduction

There are different types of membrane processes used worldwide and approximately about 20 to 30 % of them are used in food processing plants (Mohammad *et al*., 2012). Membrane processes are used due to their ability to fractionate a liquid into two liquid fractions that have different characteristics and compositions are not the same (Rosenberg, 1995). Dairy processing industry has been the leading users of filtration methods and it is estimated that almost 25 percent of Ultrafiltration (UF) membranes that are applied in food industry are utilized for milk processing (Mohammad *et al*., 2012).

UF can be defined as a pressure-driven membrane process that can be used in the separation and concentration of substances having a molecular weight between 1-10³ K Daltons (molecular size: 0.001-0.02µm) (Renner and Abd-El-Salam, 1991; Cheryan, 1998). UF has become an extremely alluring method for dairy industry since it doesn't utilize any heat processing and thus doesn't change the phase (Cheryan, 1998), as well as its application for different purposes like standardization of milk's protein content, has been recommended (Meyer et al., 2015). UF is able to adjust mass ratios of milk

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component without having any negative effects on its characteristics (Rosenberg, 1995). This process is also used to hold macromolecules and permits whey components to vary the concentration ratio because of its selective permission for low molecular component and retention of protein (Baldasso *et al*., 2011; Brans *et al*., 2004).

A solution flows under pressure over the surface of a suitably supported membrane, the solvent and certain dissolved components pass through the membrane and collected as a permeate. Depending on the characteristics of membrane used, other components of the solution are retained by membrane and concentrated, this is known as a retentate (Cheryan, 1998).

A history of cheese making using membrane commenced in the late 1960s with the invention of (MMV) process (Moubois, Macquot, Vassel). This process opened up new avenues for significant advances in cheese making including, improvement in plant efficiencies, increases in cheese yield, and development of continuous process and possibilities of creating new cheese varieties (Fox, 2003; Rosenberg, 1995; Mehaia, 2002).

Milk has complex combination of different components with wide range of sizes (1-20 nm) and high concentration of dispersed components (13 Wt. %), for fractionation membranes (Brans *et al.*, 2004). When milk is ultrafiltered there will be a significant partition of nutrients between retentate and permeate. Fat fractions, fat soluble vitamins and proteins are retained virtually completely in the retentate. (Fischbach-Greene and Potter, 1986; Glover, 1985; Renner and Abd-El-Salam, 1991). Components like soluble minerals, compounds of low molar mass and water can pass through the membrane (Bergillos-Meca *et al*., 2015; Liu *et al*., 2014). Retention of water soluble vitamins, calcium, magnesium, phosphate and trace minerals depended on the proportion bound to the protein (Fischbach-Greene and Potter, 1986).

Mineral recovery is influenced by acidification and Ca recovery is more strongly influenced by the pH at which UF is carried out than that of P recovery (Bastian *et al*., 1991). Retention of water soluble vitamins, Ca, Mg, P and trace minerals has also been depended on the proportion bounded to the protein (Fischbach-Greene and Potter, 1986). Mineral recovery is influenced by acidification. In comparison with P recovery, Ca recovery is more strongly influenced by the pH at which UF is carried out and lower pH leads to lowering mineral recovery. At high temperatures, only small amount of protein can be penetrated to permeate (Bastian *et al*., 1991), and lack of casein in permeate was shown by the researchers (Barbano *et al*., 1988).

The measured filtration characteristics were defined as follows:

$$\delta = 1 - Cp/Cb \quad \textbf{(1)}$$

- Where δ is rejection coefficient
- Cp is solute concentration in permeate.
- Cb is solute concentration in bulk solution

There are limited studies concerned with situation of milk proteins in milk during UF. Barbano *et al*. (1988) conducted a study to determine protein permeate and used SDS-Page to characterize the rejected protein percentage. Bastian *et al*. conducted a study on diffraction of milk constituents during UF and used acidified and non-acidified whole milk to determine percentage of fat, P, Ca and protein constituents in retentate and permeate (Bastian *et al*., 1991).

Although the characteristic of ultrafiltration permeates have been investigated, but to the best of our knowledge there are very limited research on the situation of all milk proteins individually against ultrafiltration which is frequently used in Iranian dairy industry companies.

The objective of this work is to characterize permeate proteins and their rejection coefficients of ultrafiltration applied in Iran Dairy Industry companies.

Materials and Methods

- *Materials*

Sodium dodecyle sulfate (SDS), acryl amide, poly acrylamide, methylene bis acrylamide, tetra methylene diamin used in this study were of analytical grade purchased from Sigama Aldrich Company (Denmark).

- *Ultrafiltration process*

Pasteurized, unhomogenized whole milk (Total solid: 11.92٪, protein: 3.195٪, fat: 3.2٪ lactose: 4.78٪ and ash: 0.712٪) was ultrafiltered at 50˚C, such that the pressure difference between two side of membrane was not more than 3.6 bar. APV ultrafiltration system had 51 spiral wounds, poly sulfone with 20000 Dalton molecular weight cut– off (DDS, Nakskov, Denmark). First and second loop had 18 membrane filters (UFPH20/6338/30FF) with 16.9 m² active surface and third loop had 15 membranes (UFPH20/6338/18FF) with 12.8 m² active surface.The UF system was run in a continuous mode at concentration factor 5X. After 120min of ultrafiltration, permeate sample was collected for further analysis. Two trail were conducted (one each of two days).

- *Electrophoresis procedure*

Milk and isolated permeate protein as describe by Barbano (1988) were diluted at 0.02 g/ml of electrophoresis sample buffer (Barbano *et al*., 1988) and were immersed in boiling water for 2 min to promote a complete sodium dodecyle sulfate and protein interaction. Sample loading was 8.5 µl per slot.

Sodium dodecyle sulfate (SDS) – polyacryleamide gel electrophoresis in 10-20٪ gradient was performed to characterize permeate and milk protein. Stacking gel was 3.75 ٪ acryl amide, 0.1٪ N, N´- methelenbis acryamide, 0.1٪sodium dodecyle sulfate, 0.075٪ ammonium per sulphate, 0.0005٪ N, N, N´, N´- tetramethlen diamin (TEMED) and 0.125 M Tris- HCl having the pH of 6.8. Separating gel was liner gradient (Akhtarian, GM-100,Iran) of 10% acryl amide plus 0.265% N, N´- methelen bis acrylamide to 20% acrylamide plus 0.535% N, N´- methelen bis acrylamide, 0.375M Tris-HCl pH = 8.8, 1%SDS, 0.038% ammonium per sulphte and 0.0003% N, N, N´, N´- tetrametheylen diamin (TEMED). Gel thickness was 1.5 mm (Akhtarian, vss1100, Iran) was as described by Verdi (Verdi *et al*., 1987).

The gradient portion of the gels was allowed to polymerized 30-45min at room temperature prior to the application. Stacking gel was polymerized after 30-45 min. Gels were maintained at 8±1˚C during electrophoresis run. A constant current of 40 mA per gel was maintained until the tracking dye entered the separating gel, then 36 mA per gel was used until the tracking dye reached the bottom edge of the gel (total running time approximately 4 h). Gels were stained 16 h in a 0.025% coomassie brilliant blue R250, 40% methanol, 7% acetic acid solution and were destained with 5% methanol,7% acetic acid solution until the background was clear. Gels were scanned with densitometer (Helena-process-24).

- *Chemical analysis*

Protein was analyzed by AOAC (991.23) methods (AOAC, 2002).

Results and Discussion

Ultrafiltration membranes reject almost all the protein from milk (Table1). This incomplete rejection of protein might be due in part to the distribution of molecular weight among the milk protein (Yan *et al*., 1979), thus some special kinds of proteins can penetrate from retentate to permeate.

In comparison with the migration

distance of protein in original milk sample and a separate multicomponent protein molecular weight standard (Figure 1), the prominent band is corresponded to α-lactalbumin. This band constitutes over 49.95±2.25% of the protein material observed by electrophoresis in each trail. These results were similar to those reported by Barbano *et al.* (1988). They reported that approximately 90% of that protein was α-lactalbumin. It is worth mentioning that this protein has been previously reported as one of the major milk proteins present in the foulant layer on polysulfone UF membrane after whey and milk ultrafiltration (Tong *et al*., 1988). Thus, because of this affinity for polysulfone UF membrane and also its molecular weight (14800 D), this protein was rejected less than other proteins in the milk (Table1). The second prominent band was β- lactoglubolin (MW: 18000). At the β-lg position on the SDS –PAGE, proteose-peptone component 5 (amino acid 1-105 and 1-107 of β- casein, MW 12158 and 12423 D, respectively, the complement of γ_2 and γ_3 casein), migrates to nearly the same position as β- lactoglubolin, if dithiothereitol (DTT) is present in the sample buffer (Verdi et al., 1984). Therefore, it is not possible to recognize each other in the SDS-PAGE. However, 22.30±2.3% protein exists on the SDS-PAGE at the 18000 Daltons molecular weight position and it was rejected 99.18±0.46% with ultrafiltration.

Table1. Average permeate and milk proteins and rejection coefficient (%) of each protein

	Permeate(w/w%)	Milk (w/w%)	Rejection coefficient (%)
Protein	0.011±0.0006	3.195±0.325	99.63±0.245
α-lactalbumin	0.00035±0.0050	0.153±0.002	96.68±1.725
β-lactoglublin	0.0033±0.0011	0.308±0.0391	99.18±0.46
Casein hydrolysis	0.0035±0.0022	0.0235±0.0175	98.39±1.005
Micelle casein	0.000	2.215±0.225	100

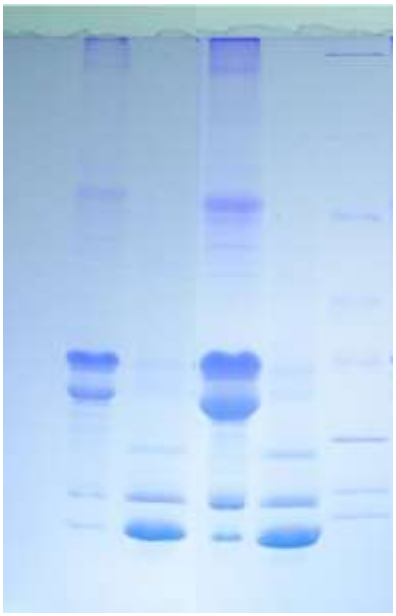

Fig. 1. The SDS-PAGE of protein isolated from UF permeate of milk and milk. Lane 1 and 3, milk. Lane 2 and 4, permeate. Lane 5, molecular weight standard (Fermentas): β-galactozidase (11600), bovine serum albumin (66200), ovalbumin (45000), lactate dehydrogenase (35000), endo nuclease Bsp981 (25000), β-lactoglublin (18400), α-lactalbumin (14400).

Proteolytic fragments of casein are usually present in low concentration in milk (Fox and Mcsweeney, 2003). This was the case for the milk used in the present study (Table 2). Such fragments are usually attributed to proteolytic damage of β-casein by somatic cell, protease, plasmin or bacterial proteases (Fox and Mcsweeney, 2003; Tong *et al*., 1988). Significant concentration of casein fragment (Table 2) was detected in the permeate protein during milk UF and they were rejected (98.39±1.005%) with ultrafiltration. Therefore, milk quality and its proteolytic activity can affect the rejection of protein. Previous studies determined the impact of milk quality on permeate flux during milk ultrafiltration (Tong *et al*., 1988).

Micelle caseins (20-300nm) were rejected completely with ultrafiltration due to their large size, thus, it cannot penetrate to permeate. The lack of caseins in analyzed permeate indicates clearly that the UF system used did not permit any physical leakage of retentate into permeate.

Table 2. The permeate protein composition

Protein	(%)[1]
α-lactalbumin	49.95±2.25
β-lactoglublin	22.30±2.3
Casein hydrolysis	30±4
Micelle casein	0.00

[1] % each protein in permeate protein which scanned by densitometer.

Conclusion

In current study we have concluded that the passage of protein through an ultrafiltration membrane pore could depend on protein molecular weight as well as other protein characteristics such as charge, hydrodynamic size, shape, hydrophobic or hydrophilic character, nature of the foulant material as well as proteolytic activity of the milk. Moreover, Incomplete rejection of casein and penetration of casein in to permeate indicates the leakage of retentate in to permeate. Finally, this paper suggests using methods that prevent the penetration of protein to UF milk that in turn leads to increasing efficacy of milk and cheese plants.

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References

AOAC. (2002). Official methods of analysis. Washangton., DC: Association of official Analytical Chemists International.

Baldsso, C., Barros, T. & Tessaro, I. (2011). Concentration and purification of whey proteins by ultrafiltration. Desalination, 278, 381-386.

Barbano, D. M., Sciancalepore, V. & Rudan. (1988). Characterization of milk protein in ultrafiltration permeate. J. Dairy Sci., 71, 2655-2657.

Bastian, E. D., Collinge, S. K. & Ernestrom, C. A. (1991). Ultrafiltration: Partitioning of milk constituent in to permeate and retentate, J. Dairy Sci. 74, 2423-2434.

Bergillos-Meca, T., Cabrera-Vique, C., Artacho, R., Moreno-Montoro, M., Navarro-Alarcon, M., Olalla, M., Gimenez, R. & Ruiz-Lopez, M. D. (2015). Influence of milk ultrafiltration on Ca, Mg, Zn and P levels in fermented goats' milk. Small Ruminant Research, 124, 95-100.

Brans, G., Schroen, C. G. P, H., Van der sman, R. G. M. & Boom, R. M. (2004). Membrane fractionation of milk: state of the art and challenges. J. Membrane Sci. 243, 263-272.

Cheryan, M. (1998). Ultrafiltration and microfiltration handbook. Technomic publishing company. Pennsylvania.

Fischbach-Green, L. & Potter, N. (1984). Effect of ultrafiltration on retention of minerals and other components of milk. J. Food Sci., 51, 345-347.

Fox, P. F. & Mcsweeney, P. L. H. (2003). Advanced Dairy Chemostry_1 Proteins 3rd Edition. Part A. Kluwer Academic.

Glover, F. A. (1985). Ultrafiltration and Reverse Osmosis for the Dairy Industry, Technical bulletin 5. England.

Liu, D. Z., Weeks, M. G., Dunstan, D. E. & Martin, G. J. (2014). Alterations to the composition of casein micelles and retentate serum during ultrafiltration of skim milk at 10 and 40 C. Int. Dairy J. 35, 63-69.

Mehaia, M. A. (2002). Manufacture of fresh soft white cheese (Domiati-type) from ultrafiltered goats' milk, Food Chem. 79, 445-452.

Meyer, P., Mayer, A. & Kulozik, U. (2015). High concentration of skim milk proteins by ultrafiltration: characterisation of a dynamic membrane system with a rotating membrane in comparison with a spiral wound membrane. Int. Dairy J. 51, 75-83.

Mohammad, A. W., Ng, C. Y., Lim, Y. P. & Ng, G. H. (2012). Ultrafiltration in food processing industry: review on application, membrane fouling, and fouling control. Food Bioprocess Tech., 5, 1143-1156.

Renner, E. & Abd el-salam, M. H. (1991). Application of ultrafiltration in the dairy industry. Elsevier Applied Science.

Rosenberg, M. (1995). Current and future application for membrane processes in dairy industry, Trends Food Sci. Technol. 6, 12-19.

Verdi, R. J., Barbano, D. M., Dellavalle, M, E. & Senky, G, F. (1984). Variability in true protein, casein non protein nitrogen, and proteolysis in high and low somatic cell milks. J. Dairy Sci., 71, 230-242.