Microfiltration-based separation of milk proteins and their enzymatic hydrolysis using papain

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1. INTRODUCTION

Milk and dairy products contain a wide range of macro and micronutrients that are essential for maintaining overall health and supporting balanced growth. Milk proteins are listed among the “big 8” allergens, due to the presence of linear and conformational epitopes. The food allergies (FAs) can be divided into two main categories like immunoglobulin (Ig)E-mediated or non-IgE mediated. Future of milk allergen reduction may lie in enzymatic hydrolysis by the targeting of the allergenic epitopes. In food industry, papain is used to produce different protein hydrolysates. Bioactive peptides are biologically active molecules that remain closed within parent proteins and are released upon protein cleavage. Microfiltration (MF) can be applied as a pre-treatment to separate casein micelles from milk serum proteins (SP) to produce a casein-enriched retentate, while the retentate with enzyme treatment seems possible candidate as a hypoallergenic dairy drink product.

2. MATERIALS AND METHODS

Protein separation of ultraheat-treated skimmed milk were performed by a Membralox tubular microfiltration membranes with active filtration area 5×10^{-3} m^2 and pore size 5 µm, 1.4 µm, and 0.8 µm (Pall Corporation, Crailsheim, Germany), placed in a stainless steel-made cross-flow membrane module. A static turbulence promoter – a double helix ribbon - made of stainless steel (SS316) was fit in the lumen side of the membrane and was used during each membrane filtration experiment. Enzymatic hydrolysis of Cow milk proteins was performed using papain enzyme produced by HIMEDIA®. During the experiments, a temperature of 50 °C was selected from the activation temperature range to prevent heat-induced precipitation of the thermosensitive milk proteins. After hydrolysis was completed, the enzyme was inactivated by heating to 70 °C for 30 min.

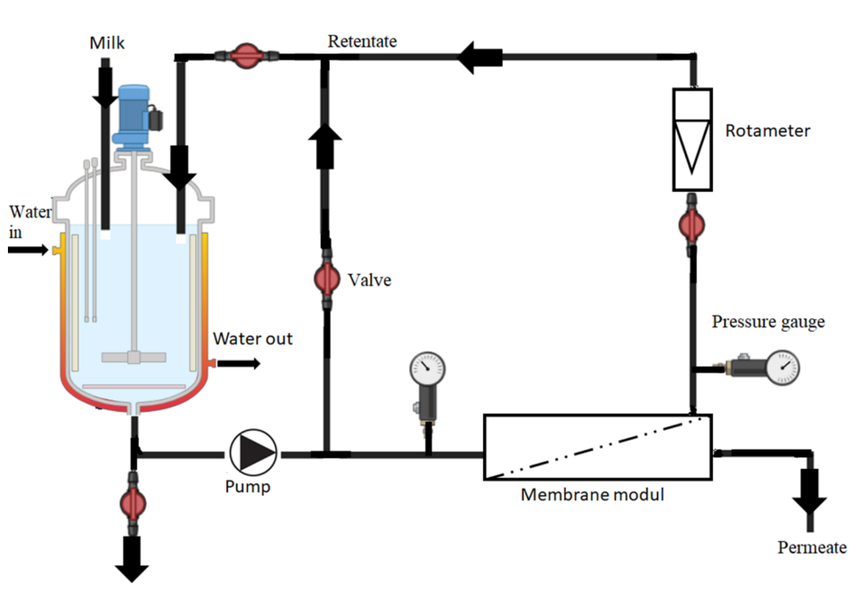

Figure 1.: Flow sheet of the cross-flow membrane module

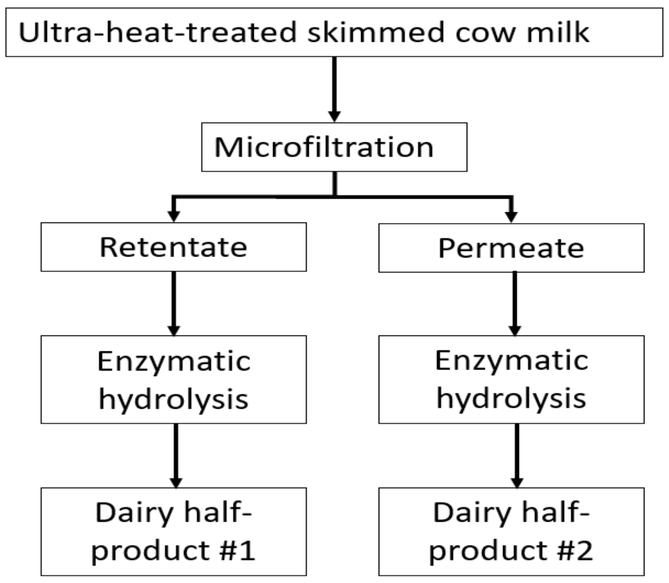

Figure 2.: Process flowsheet for MF separation and hydrolysis of milk proteins

3. RESULTS AND DISCUSSION

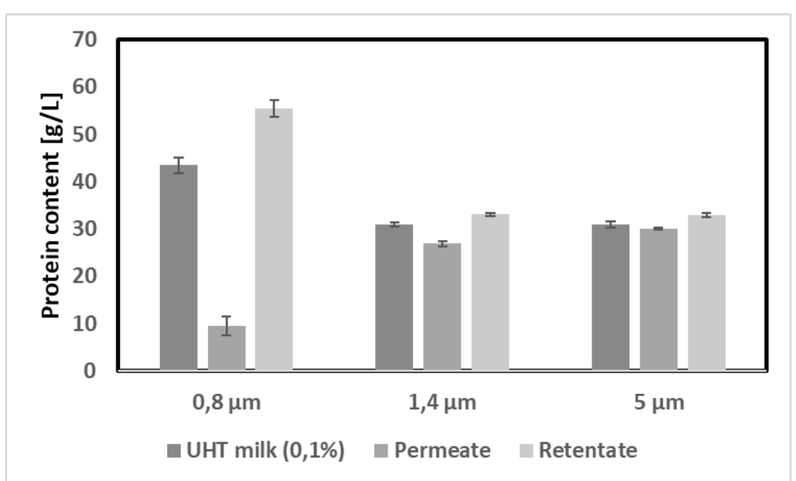

Figure 3.: Concentration of protein in the feed,permeate and retentate side of the MF membranes

Concentration of protein in the retentate and permeate were not changed significantly (26.9 g/L and 33.09 g/L) during the filtration with the 1.4 membrane which refers to an overall protein retention (R%) of 13,2%. In the case of the 5 µm membrane, the permeate (30.09 g/L) and retentate (33.04 g/L) protein contents are very similar to the feed (31.0 g/L), indicating only negligible protein separation. On the contrary, in case of the 0.8 µm membrane, a significant reduction in protein content is observed in the permeate (9.5 g/L), indicating a high retention efficiency (R% = 69,4) and due to this fact the retentate had a high protein concentration (55.5 g/L).

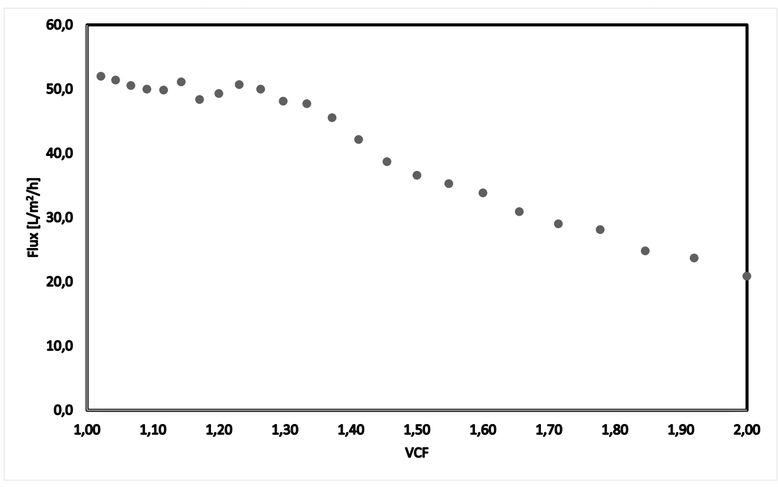

Figure 4.: Permeate flux during membrane filtration of UHT milk (0.8 µm)

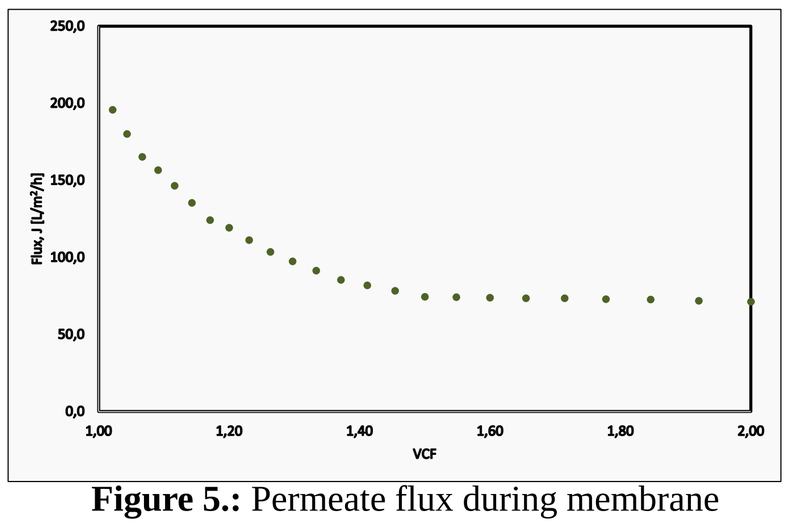

Figure 5.: Permeate flux during membrane filtration of UHT milk (1.4 µm)

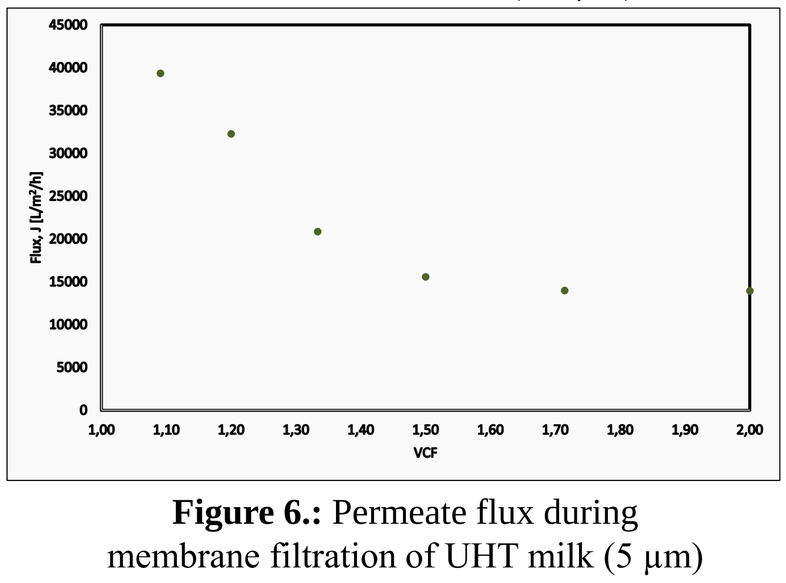

Figure 6.: Permeate flux during membrane filtration of UHT milk (5 µm)

It was observed that permeate flux was decreased with increase of the VCF. With increase of VCF, permeate flux was reduced due to formation of concentration polarization on membrane surface. In Fig. 5, it is shown that the initial value of permeate flux was 195 L m−2·h−1 which was declined to 71 L m$^{-2.h-1}$ when VCF 2 was reached. In Fig. 4, it is shown that the initial value of permeate flux was 52 L m$^{-2.h-1}$ which was declined to 21 L m$^{-2.h-1}$ when VCF reach to 2. The reduction of the permeate flux was 63.5 % and 59.6% with the 1.4 and 0.8 µm membrane.

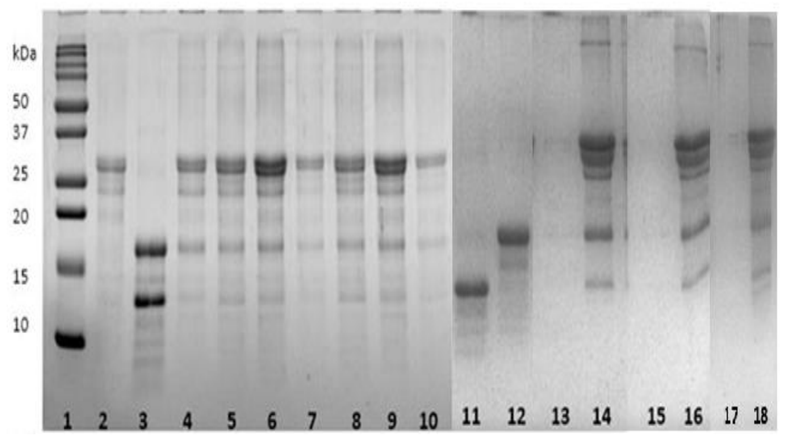

Figure 7.:SDS-PAGE patterns of the membrane separated and enzymatic treared milk proteins; (lane 1: molecular weight marker, lane 2: casein, lane 3: α-lactalbumin+β-lactoglobulin, lane 4: UHT milk, lane 5: UHT milk hydrolyzed by papain /0,016 g/200 ml/, lane 6: retentate /1.4 µm/ hydrolyzed by papain /0,016 g/200 ml/, lane 7: permeate /1.4 µm/ hydrolyzed by papain /0,016 g/200 ml/, lane 8: UHT milk hydrolyzed by papain /0,024 g/200 ml/, lane 9: retentate /1.4 µm/ hydrolyzed by papain /0,024 g/200 ml/, lane 10: permeate /1.4 µm/ hydrolyzed by papain /0,024 g/200 ml/, lane 11: α-lactalbumin, lane 12: β-lactoglobulin, lane13: permeate /0.8 µm/, lane 14: retentate /0.8 µm/, lane 15: permeate /0.8 µm/ hydrolyzed by papain /0,016 g/200 ml/, lane 16: retentate /1.4 µm/ hydrolyzed by papain /0,016 g/200 ml/, lane 17: permeate /1.4 µm/ hydrolyzed by papain /0,024 g/200 ml/, lane 18: retentate /1.4 µm/ hydrolyzed by papain /0,024 g/200 ml/).

Comparing figure Fig 7. it can be observed that the 0.8 µm membrane separates the milk proteins with much more efficiency than the membrane with 1.4 µm pore size, which is consistent with the results of the total protein content determination. In the enzymatically treated permeate fractions of the 0.8 µm membrane, casein and whey proteins can only be detected in traces at both lower and higher papain concentrations (lane 15 and lane 17). In case of other samples the proteolysis could reduce the molecular size what can be observed in lane 5 and 8 when comparing them to lane 4 (feed).

4. CONCLUSION

The analysis of the microfiltration of fat free UHT milk with ceramic membranes suggested pore size 0.8 µm in order to fractionate the proteins of the feed with satisfying high throughput. This membrane could reduce the permeate’s protein content below 10 g/L ($R_{protein}$% = 69.4). In case of the feed and the retentate of the 0.8 µm membrane as well in all samples of the 1.4 µm membrane the enzyme treatment couldn’t hydrolyse the total amount of proteins with the applied parameters. The enzymatic treatment of the raw material and the separated streams found to be outstanding effective on the permeate samples of the 0.8 µm membrane. The low molecular peptides have to be separated from such hydrolysates with another following unit process (e.g. MF/UF) if hypoallergenicity is a requirement in the final product.

5. REFERENCES

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