Optimization of microfiltration for separation of whey proteins

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Abstract

Microfiltration is a low pressure-driven membrane filtration process, which is based on a membrane with an open structure allowing dissolved components to pass while most non-dissolved components are rejected by the membrane. Microfiltration is defined to separate in the range 0.5 to 5µm, which includes viruses, bacteria, etc. In the dairy industry, microfiltration is widely used for bacteria reduction and fat removal in milk and whey as well as for protein and casein standardization. The purpose of microfiltration membrane testing was to optimize whey filtration processes, with the aim of achieving high permeability for whey proteins (especially lactoferrin) and retention of microorganisms and other whey components that would limit the use of whey in further processing operations. The most efficient mode of microfiltration ceramic membrane operation was established as well as the cleaning regime. The highest lactoferrin permeability was 51 % at a pressure of 1 bar. Membrane was effectively cleaned in three steps: first using 0.4 % sodium hydroxide solution (NaOH) followed by 0.3% nitric acid solution (HNO_3) and 0.5 % sodium hypochlorite solution (NaOCl) at 60 °C and at a higher pump speed than the operating speed and at the pressure of reverse flow at 0.5 bars.

Introduction

Currently, there is growing interest in new applications of whey and its derivatives for various food products with improved quality that are beneficial to health (i.e., as functional foods or nutraceutic products). The drug and food industries have a great interest in developing products using whey and its derivatives as raw material sources.
Some of the most important components of whey are lactose and soluble proteins. Typically, cheese whey contains 5–6% lactose, 0.8–1% protein and 0.06% fat. It is one of the most valuable biological sources of proteins (representing about 20% total proteins in milk), which have attracted interest for potential use in human diet products with specific functional properties (de Souza *et al*, 2010).
Microfiltration (MF) is a widely used process with many applications in food industry. Ceramic membranes are the only ones that satisfy all the requirements of the applications in the dairy industry i.e. a strong mechanical resistance which allows the use of high recirculation velocities of viscous MF retentates, a wide tolerance to pH (0.5 to 13.5) allowing its usage for cleaning in place of caustic soda (up to 3%), of nitric acid (up to 2%) – but phosphoric and hydrofluoric acids should be avoided – and of sodium hypochlorite for sanitation .
The main problem with MF is fouling, which leads to reduce permeate flux and membrane selectivity. Protein adsorption in the internal structure of the pores has been reported as the most

dominant step in fouling phenomena by some researchers (Bowen and Gan, 1991), while others suppose that flux decline results mostly from surface deposition of a cake including protein aggregates (Jim *et al.,* 1992). Many researchers proved that there is a transition in the fouling mechanism. Zokaee *et al*. (Zokaee Ashtiani *et al.*, 1999) reported a shift from the internal blocking to cake formation during a continuous membrane filtration of a biological solution.
In this study, the effects of operating conditions, such as the operating pressure and fluid velocity, on each individual resistance in the steady-state crossflow microfiltration of whey were investigated using ceramic tubular membrane (pore size 0.5 μm).

Materials and methods

Table 1. Specifications of MF ceramic membrane with pore diameter 0.5 μm.

Specifications of MF ceramic membrane	
Model	CMF19040
Material	α-Al_2O_3/ZrO_2
Diameter of pore	0.5 μm
Number of channels	19
Diameter of membrane	30 mm
Length of membrane	1016 mm
Construction	multichannel tube membrane
The surface of the membrane	0.24 m^2

Filtration
First, we determined the maximum pressures that can be achieved at a given pump speed at each membrane used. Before each batch of whey filtration, water flow (Baseline 1) was measured to determine the degree of membrane cleaning. When a constant permeate flow was detected, we reached a steady state. After filtration of the whey, the system was washed with water and the water flow was measured again (Baseline 2). Then, the membrane was washed from the permeate and retentate sides, performed the backwash of water and the filtration of water. This was followed by measuring the water flow after cleaning the membrane with water (Baseline 3). After chemical cleaning, the final water flow (Baseline 4) was measured and compared to the initial water flow (Baseline 1). Water flow measurement procedures are required to determine the membrane cleanliness status.

Table 2. Filtration procedure.

Procedure	Description	Quantity (L)	Temperature (°C)	Resistance
Baseline 1	Initial water flux	30	25	R_m
Acid Whey filtration	Determination of whey flux	10	25	R_t
Cleaning the system with water	Flushing and cleaning the system with water	120	25	/
Baseline 2	Determination of water flux	30	25	/
Cleaning the system with water	Cleaning of the system with water (back flow,	80	25	/

	washing of membrane from permeate and retentate side and flow of water from retentate to permeate side)			
Baseline 3	Measurement of water flow	30	25	R_{irev}
Chemical cleaning	Cleaning the system with a cleaning agent	10	50-60	/
Baseline 4	Measurement of water flow	30	25	/

Membrane cleaning

The system was cleaned at 80% pump speed and 0.5 bar of pressure. An experiment was made to clean the membrane with different concentrations of cleaning agents and different operating conditions (Table 3). The most effective cleaning procedure was determined and used.

Table 3. Cleaning agents and conditions during membrane cleaning.

Cleaning agent	Concentration (mas %)	pH	Temperature (°C)
NaOH	0,4 - 1	11,5	50 - 60
HNO_3	0,3 - 1	1,3	50 - 60
NaClO	0,5	7,8	50 - 60
Divos 120CL	1	10	50

NaOH was supplied by Honeywell Fluka, Germany; HNO_3 by Fluka Riedel-de Haën, Germany; NaClO by Varikina, Sidap, Italia and Divos 120CL by Diversey, Netherlands.

Ion exchange chromatography for the detection and quantification of LF

High-performance liquid chromatography (HPLC) is the most widely used separation method and is used in the analysis of whey proteins. HPLC separates proteins by their polarity, where at high pressure the proteins are adsorbed onto the stationary phase of a column filled with small polar particles (e.g. silica). The polar parts of the proteins interact with the polar surface of the column, and the most polar proteins are secreted with the mobile phase (Deeth & Bansal, 2019). In cation exchange chromatography, LF binds to negatively charged groups, bounded at matrix in the column, via positively charged basic amino acids on its surface. LF has a positive charge at neutral pH, which can be used for its separation and analysis (Deeth & Bansal, 2019). Whey samples were analyzed by cation exchange chromatography using a CIMac ™ COOH-0.1 analytical column (BIA Separations, Slovenia) and a Shimadzu UFLC HPLC system (Japan) with a diode array detector. The column was equilibrated for two minutes in mobile phase A (25 mM sodium phosphate (Na_3PO_4), pH 7.5). Then, using an automatic sampler, 15 µl or 25 µl of whey sample was previously applied to the column, which was pre-filtered with 0.22 µm CA filters (Sartorius, Germany). The column was then washed for 0.5 min with mobile phase A, then eluting the bound proteins with a linear gradient of mobile phase B (2M NaCl in 25 mM Na_3PO_4, pH 7.5) to 62.5% B in 2.5 min. A phase B mobile phase gradient of up to 100% in 0.5 min followed by column wash with 100% B for 1 min, followed by a gradient of up to 100% A in 0.5 min and column equilibration in 100% A for 2 min before analysis of the following sample. The flow rate during analysis was 1 ml / min. The absorbance was measured

at 226 nm. The chromatograms were analyzed with Postrun (Shimadzu). The peak areas with the retention times corresponding to the retention times of the LF standard were integrated. The calibration curve was prepared by analyzing the various solutions of the LF standard, using which, from the peak surfaces in the whey samples analyzed, the concentration of LF in the samples was calculated.

Results and discussion

Prior to each whey filtration experiment, the clean water permeate flux was measured in order to determine native membrane resistance under each operating condition (Fig.1) and degree of membrane cleaning after the membrane has been used. The graph is called Baseline 1.

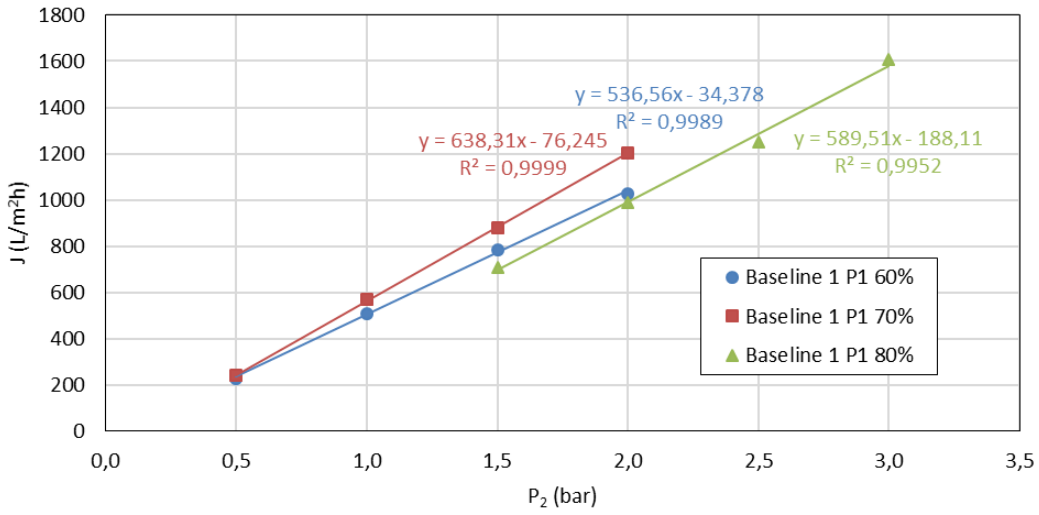

Figure 1. Water flux at different operating pressure in order to determine Baseline 1.

Baseline 1 is the initial flow of water, determined based on maximum retentate pressures and different pump speeds from the retentate side of the vessel, across the ceramic membrane into the permeate side of the vessel. We determined three different pump speeds (60%, 70% and 80%) and four different retentate pressures at each pump speed. Baseline 1 had to be specified because the same conditions were used to filter the whey later on. This had to be adjusted so that later in the steps we could identify the membrane fouling where it was important to operate under the same conditions. For each pressure used (from 0.5 to 3 bar), it was important to achieve a constant flux (steady state), which was measured for ten minutes.

Whey filtration

Whey filtration was performed from the retentate side of the tank, through the ceramic membrane into the permeate side of the tank, by increasing the retentate pressure at a specified pump speed. We filtered 10 L of acidic whey at its pH (4.8) and 60% of the pump speed according to the following procedure (Fig.2):

1. Filtration was started at a retentate pressure of 0.5 bar and when steady state (15 min) was reached, the first permeate sample was taken.

2. Retentate pressure was increased and filtration continued at 1 bar. After 15 min of steady state, a permeate sample was taken.
3. Retentate pressure was increased and filtration continued at 1.5 bar. After 15 min of steady state, a permeate sample was taken.
4. Retentate pressure was increased and filtration continued at 2 bar. After 15 min of steady state, a permeate sample was taken

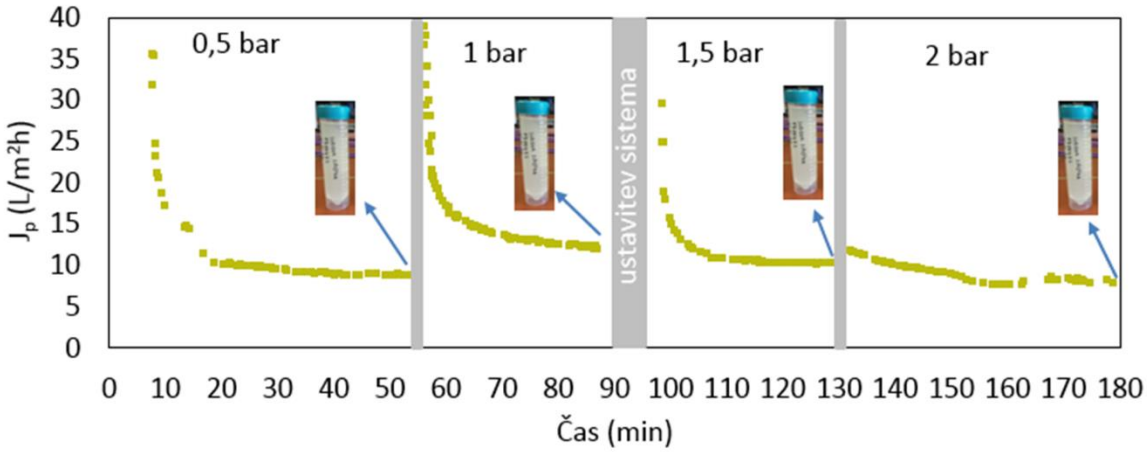

Figure 2. Whey flux at different pressures.

Initially, a rapid decrease in flow is observed in the first minutes of filtration due to concentration polarization effect. The flux decline could be classified into two distinct phases in this figure. “Phase I” and “Phase II” separated the sharp flux reduction in the first 5-10 minutes of the process from the slow reduction up to the 20th minute and the steady-state condition after that.

Baseline 2 represented the flux of water after flushing the system with water where the pores of the membranes were not cleaned. The retentate and permeate side of the vessel, the tubes in the system, and the outside of the membrane were cleaned. Baseline 2 was determined under the same conditions as Baseline 1 at 60% pump speed and flux rates at retentate pressure of 0.5 bar, 1 bar, 1.5 bar and 2 bar were measured. Baseline 3 was the flow of water after cleaning the pore membrane with a water backflush. The return water flow was performed by reversing the flow from the permeate side of the vessel through the pores of the membrane to the retentate side of the vessel. The procedure was repeated and water was changed until clean water flowed into the retentate side of the vessel. Baseline 4 presented the water flux after chemical cleaning. Baselines from 1-4 are presented on Fig. 3.

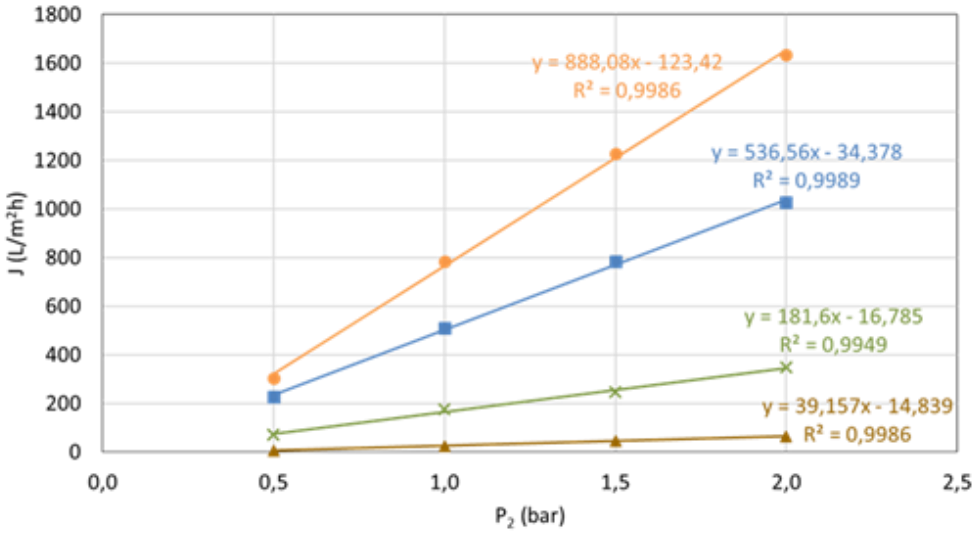

Figure 3. Water flux at different operating pressure in order to determine Baseline 1-4.

Determination of fouling - The "*Resistance-in-Series*" model is commonly used to study the role of resistances from different fouling mechanisms. Therefore, the part played by the internal and external fouling in the membranes could be better understood. These resistances include membrane resistance, an external or reversible fouling R_{rf} (m^{-1}) which consists of cake layer deposition and concentration polarization, and irreversible resistance, R_{if} (m^{-1}). The latter is due to particle and macromolecule deposition and adsorption in the membrane pores.

As expected, the membrane resistance for a special and fixed membrane morphology is independent of the fouling and is a function of compaction for polymeric membranes (Rezaei *et al.*, 2010).

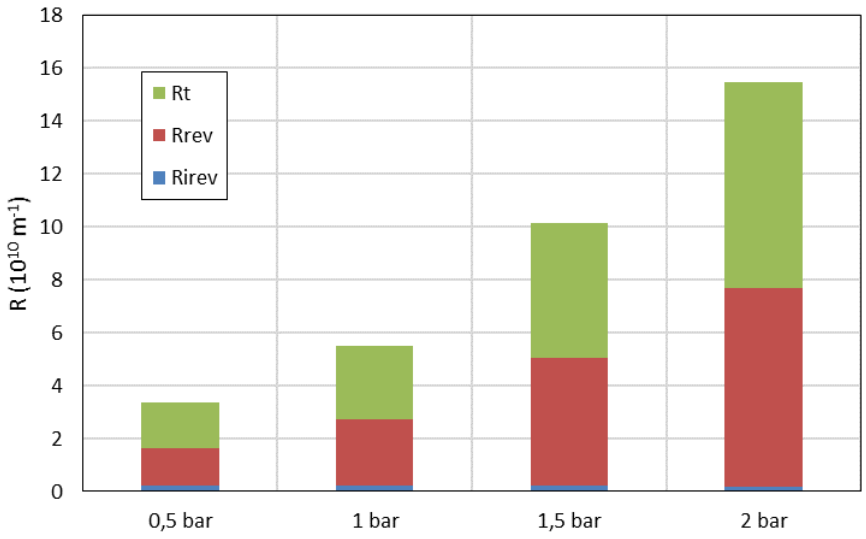

Figure 4. Total resistance, resistance of reversible and irreversible fouling of MF ceramic membrane.

Concentration of the lactoferrin was calculated and it is presented in Table 4.

Table 4. Concentration of the lactoferrin (LF).

t (min)	P_2 (bar)	$c_{LF,P}$ (mg/L)	P_{LF} (%)	R_{LF} (%)
0	0	47,6	/	/
50	0,5	4,9	10	90
85	1	24,2	51	49
130	1,5	19,8	42	58
180	2	23,2	49	51

From the Table 4 is seen that most of the lactoferrin passed into the permeate at a pressure of 1 bar after 90 min.

Conclusion

Whey is a by-product of the dairy industry and is produced during cheese production. Whey removal is a major environmental problem in the dairy industry. Whey proteins, because of their composition, have valuable physicochemical properties that can be useful. It has many positive health effects such as antimicrobial, antibacterial, anti-carcinogenic properties and improves immune resistance. The overall resistance of filtering was increased because the pore clogging process is dominant. The resistance of reversible clogging increases with increasing pressure. Reversible fouling is mainly associated with the formation of a filter cake or gel. At higher pressure, the greater the effect of achieving irreversible clogging, which cannot be eliminated by the use of backwash of water. Irreversible clogging is related to clogging of pores, which take place more rapidly at higher pressures. The reason for reversible clogging is the poorly bound proteins within the pores and the formation of protein layers on the membrane surface.

Reference

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