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Research Paper

Effect of Temperature and Module Configuration on Membrane Fouling and End-Product Quality of Acidic Whey using Ceramic Ultrafiltration Membrane

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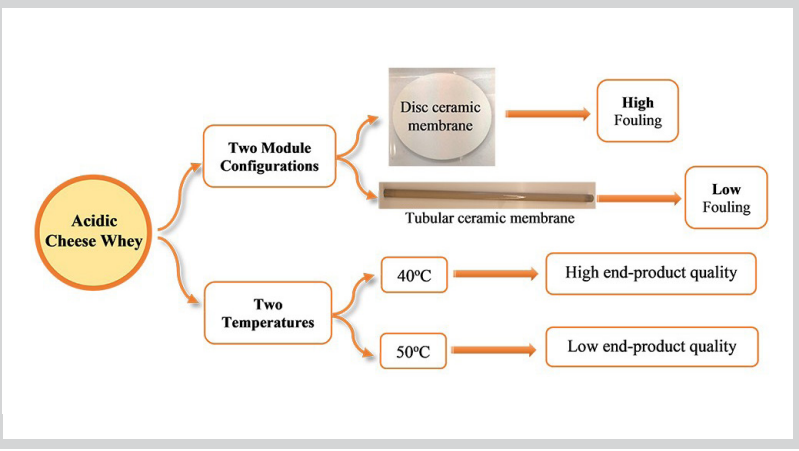
Highlights

- Effect of whey temperature and module configuration on membrane performance.
- MFI model was used to evaluate the fouling mechanism.
- Fouling was higher in the disc membrane module comparing to the tubular one.
- Feed temperature affects the end-product quality.
- Antibiotics, hormones, and heavy metals were found in very low concentrations.

Graphical abstract



Abstract

Ceramic membranes have been used in different dairy industry processing owing to their food compatibility, high stability to temperature and pH, and high fractionation efficiency. This work aims to optimize the performance of ceramic ultrafiltration membrane for acidic whey processing based on the filtration temperature and module configuration. Disc and tubular membrane modules were used with a ceramic membrane 15 kDa molecular weight cut-off and at whey temperature of 40 °C and 50 °C for both modules. The filtration performance was evaluated with normalized flux and membrane fouling index model. The end-product quality was monitored by analyzing protein, lactose, antibiotics, hormones, and heavy metals. It was found that the module configuration has a great effect on flux behavior and membrane fouling. The tubular module shows better performance with regard to normalized flux and membrane fouling index. However, at a higher temperature, the membrane fouling was higher with the disc membrane module and lower with the tubular one. In terms of end-product quality, the whey temperature is affecting protein and lactose concentration while the module configuration did not show a significant effect. Antibiotics, hormones, and heavy metals were found in concentrations that do not affect human health.

1. Introduction

Whey is a by-product of cheese making process and it has high nutritional value. It mainly comprises protein and lactose as well as fewer amounts of minerals and fat [1]. Whey protein is one of the main components of cheese whey. There are two types of whey according to their original pH which are sweet whey (pH=5.6 to 7) and acid whey (pH<5) [2,3]. Protein is one of the main components of whey. Whey protein consists mainly of α-Lactalbumin (α-La), β-Lactoglobulin (β-Lg), and proteose-peptone which are representing 48%, 19%, and 20% of cheese whey proteins respectively while the bovine serum albumin (BSA) and Immunoglobulin represent the rest [4]. The molecular weight of β-Lg, α-La, proteose-peptone, BSA, and Immunoglobulin are (18.2 – 36.9) kDa, 14.2 kDa, (4 – 80) kDa, (66 – 69) kDa, and (150 – 1000) kDa respectively [4-7]. On the other hand, cheese whey should have a permissible level of antibiotics, hormones, and heavy metals, however, the produced whey from cheese processes might be contaminated with such impurities. Antibiotics (e.g. penicillin, cephalosporin, macrolide, sulphonamide) were detected in the cow's milk due to their excessive use in the treatment of infectious diseases in livestock and consequently, could be transported to the whey [8]. The accumulation of hormones in the milk then to the milk products can cause various health problems to the humans such as in initiation and provoke of the prostate, breast, and endometrial tumors [9]. Besides, milk and milk products might contain different types of heavy metals which have been detected in milk and consequently in whey due to the new technologies applied in dairy industries [10].

Pressure-driven membrane process is one of the highly applicable alternatives for the separation of whey components. These processes,

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including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) are preferred in whey fractionation and also recovery of valuable ingredients [11-13]. UF is one of the common membrane processes for whey fractionation by retaining the protein in the concentrate stream while the lactose and minerals are passing to the permeate stream [14-16]. However, some operational conditions can affect the separation performance such as whey temperature and module configuration. According to different literature studies, the polymeric membranes are easily affected by changing the operating parameters like temperature and pH which can cause operating problems, such as fouling, during acidic whey filtration. For that reason, the ceramic membranes present more operational advantages like their high resistance to low pH and high temperature than the polymeric membranes [17].

Temperature is one of the main operating conditions to be controlled during the membrane filtration in dairy industries because of the changes in the behavior of protein molecules [18,19]. Increasing the operating temperature can cause the denaturation of protein in suspension and so the protein molecules adsorption on the membrane surface during whey processing [6,20]. β-lactoglobulin proteins exposed to a small conformational change above 40ºC. Furthermore, the increase of whey temperature between 50ºC to 60ºC causes unfolding of the protein [6]. Kuo and Cheryan [21] used a polysulfone UF membrane having a molecular weight cut-off (MWCO) as 20kDa in a spiral-wound module. They used that module for acidic whey filtration and they found that the flux values increased with increasing temperature [21]. Contrary to this, Barukčić et al. [22] used a ceramic membrane having 20kDa of MWCO and indicated that the permeate flux of the ceramic membrane was higher at 20ºC compared to 50ºC.

The other important parameter that influencing the membrane filtration performance is the module configuration. The module configuration such as tubular or disc affects the feed flow direction and streamlines across the membrane surface and so the membrane fouling mechanism and end-product quality. The fouling occurrence can be decelerated by choosing a suitable module type [11]. Especially, the cross-flow filtration mode decreases the membrane fouling problem by minimizing the cake layer [23]. Bhattacharjee et al. [24] explained this phenomenon in tubular membrane modules with the effect of the turbulent flow regime. This enhances the solute diffusion from the membrane surface to the bulk side and so causes the less compact cake on the membrane surface and high permeate flux.

This study is aiming to assess the effect of module configuration and feed temperatures on the performance of the ceramic UF membrane. The permeate flux, end-product quality, and fouling mechanisms were investigated. Disc and tubular UF ceramic membranes having a MWCO of 15 kDa were used. The temperature of the cheese whey was set at 40ºC and 50ºC for each filtration experiment. The quality of the end-product was investigated in terms of protein content, lactose content, antibiotics, hormones, and heavy metals.

2. Materials and Methods

2.1. Ceramic ultrafiltration membranes

The whey filtration was conducted with two different geometries of commercial ceramic membranes; disc and tubular membrane. Both membranes have a MWCO of 15kDa and they were obtained from Sterlitech Corporation (WA, USA). The active and support layers for both membranes are made of Zirconia (ZrO_2) and Titania (TiO_2), respectively. The length of the tubular ceramic membrane is 25 cm. Table 1 illustrates the properties related to the membranes used in this study.

2.2. Whey properties

A real-scale manufacturer of milk powder in Turkey has provided the whey used in this experiment. The whey was kept at 4ºC before handling. It was centrifuged and the fat was extracted before using it in the membrane filtration system. The properties of whey are illustrated in Table 2. The pH and electrical conductivity were measured by WTW series Inolab pH-EC meter. Lactostar device (Funke Gerber, Germany) was used for the characterization of raw whey components (e.g. total solids, protein, lactose, and minerals).

2.3. Experimental system

The experimental system is shown in Figure 1. A hot plate stirrer (Heidolph, Hei-Tec) was used for mixing and heating the feed. The peristaltic pump (Filttec, FPP-1) with pump head (Filttec, PH-II3) is pumping the feed to the membrane module. The pressure was controlled by regulating the pressure valve. A precision scale (AND, FX-5000i) was used for data collection of the permeate volume by connecting the scale to a personal computer. Two separated membrane systems were used (tubular and disc) and their flow regime are demonstrated in Figure 2a,b respectively.

2.4. Filtration procedure

The operating temperature of the acidic whey was set at two different temperatures (40ºC and 50ºC). The selection of temperature was followed by considering the conformational change of β-lactoglobulin which occurs above 40ºC. The temperature was maintained at a constant temperature (±2ºC) by using the hot plate. The cross-flow velocity by using a peristaltic pump for the disc and tubular membrane module was 0.2 ± 0.03 m/s. The permeate was collected in a separate beaker and the concentrate was recirculated to the feed tank. The samples were acquired from permeate, concentrate, and feed streams and were kept at 4ºC before being analyzed. The permeate volume was collected on digital balance and the data were recorded within the one-minute interval by using Win-CT software. Equation 1 below was applied to calculate the flux values.

$$J = ((\Delta m/\rho)/1000)/(A * (\Delta t/60)) \quad (1)$$

where, J is the permeate flux of whey filtration ($L/m^2.h$), Δm is the mass of permeate collected (g) at a specific time interval, ρ is the feed density (g/cm^3), A is the membrane active filtration area (m^2), and Δt is the time interval (min). At each filtration cycle, the same membrane was used at the transition from 40ºC to 50ºC after chemical cleaning. For observing the flux change trend, the flux values (J) at the whey filtration cycle were recorded with filtration time and each flux value was correlated with the distilled water flux value (J_w) at the beginning of the filtration cycle. So the ratio between J and J_w is called normalized flux (J/J_w). The filtration was continued until filtering 50% of the feed (i.e. VCF=2).

2.5. Fouling experiment

The fouling parameters were calculated with the membrane fouling index (MFI) model. MFI is determined from the slope value of the linear relation with t/V_s (s/m) versus V_s(m). The parameter t is time (s) and V_s is the cumulative permeate volume per membrane surface area, (m^3/m^2). Equation 2 was used to calculate the specific cake resistance [25].

Table 1
Properties of commercial disc and tubular ceramic UF membranes.

Properties	Disc Membrane	Tubular Membrane
Membrane thickness (mm)	2.5	2.0
Outer diameter (mm)	90	10.0
Inner diameter (mm)	-	6.0
Maximum filtration area (cm^2)	52.8	40.0
Pure water permeability @25ºC ($L/m^2.h.bar$)	43	71

Table 2
Acidic whey properties.

Parameters	Unit	Average
pH	-	4.7±0.1
Electrical Conductivity (EC)	mS/cm	8.2±1.6
Total Solids (TS)*	%	8.1±0.4
Protein*	%	3.0±0.2
Lactose*	%	4.4±0.3
Minerals*	%	1.2±0.2

*These parameters were measured with Lactostar device.

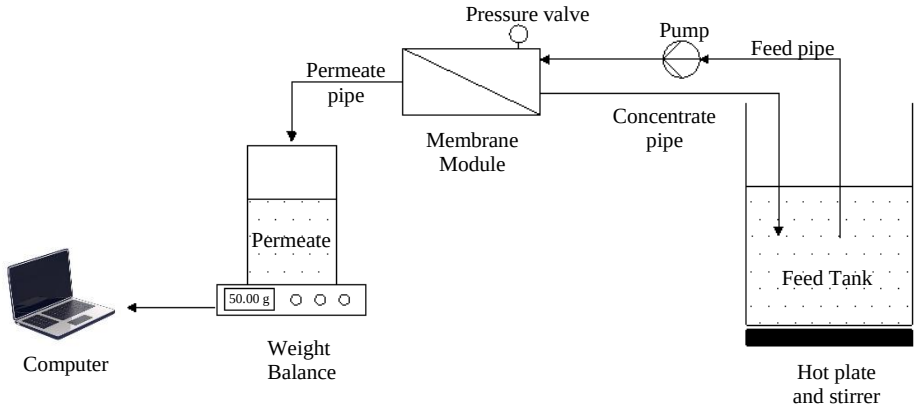


Fig. 1. Experimental setup.

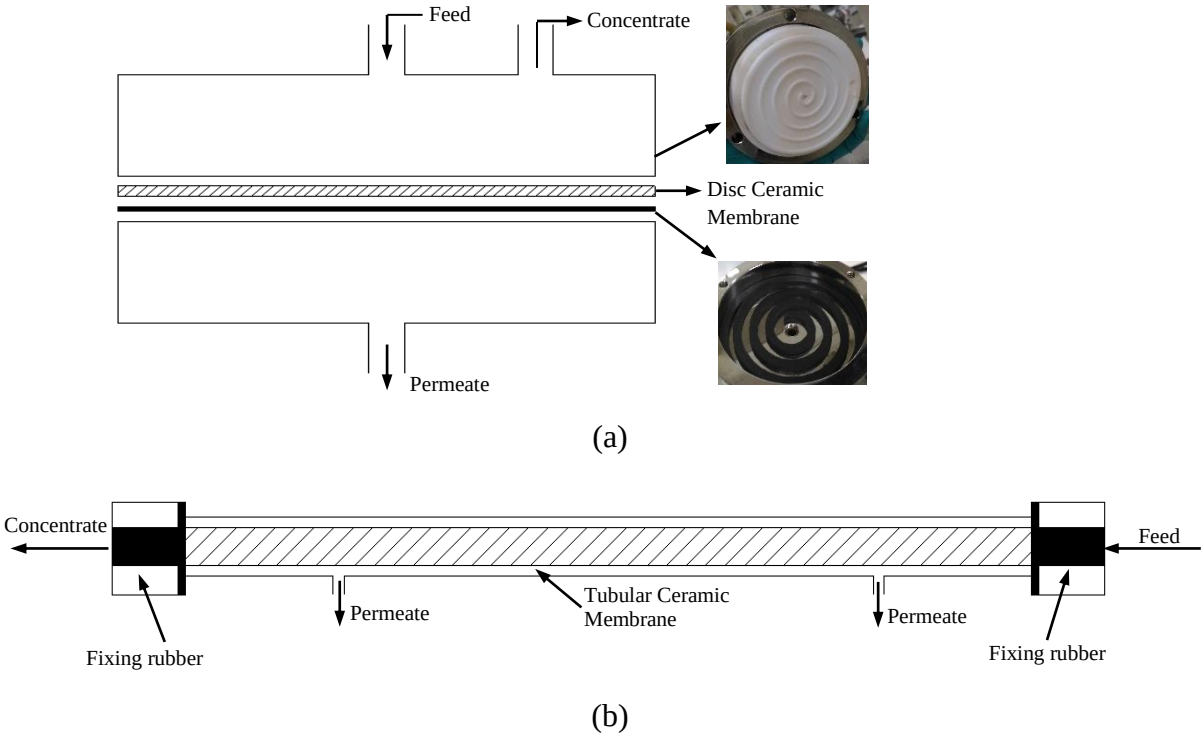


Fig. 2. (a) Disc membrane system, and (b) tubular membrane system.

$$MFI = (\alpha * \mu * C_{TS})/(2 * \Delta P) \quad (2)$$

where α is the specific cake resistance (m/kg), μ is dynamic viscosity (Pa.s), C_{TS} is the total solids concentration in feed (mg/L), and ΔP is transmembrane pressure (kPa).

The chemical cleaning procedure of the fouled ceramic membranes of both disc and tubular was conducted in two steps by using Sodium Hydroxide (NaOH) and Phosphoric acid (H_3PO_4 (85%)). Firstly, the base cleaning was applied for the removal of organic substances by using 0.1 M and 0.4 M NaOH solution for disc and tubular membrane respectively for 30 min. at 85ºC. Afterward, the membranes were rinsed by using distilled water for pH neutralization. Secondly, acid cleaning was applied for inorganic substances removal by using 85% H_3PO_4 solution with 0.013 M for 15 min. at 50ºC followed by washing the membranes with distilled water. The chemically cleaned ceramic membranes should be stored in distilled water overnight before using them in the new filtration cycle.

To check chemical cleaning performance, the distilled water was filtrated for both membranes (disc and tubular) for 30 min. at room temperature (25ºC). The distilled water flux of the bare and chemically cleaned membrane was calculated. The flux recovery rate was calculated relying on flux results after the cleaning the fouled membranes chemically by using the equation below:

$$Flux\ recovery\ rate\ (\%) = (J_c/J_w) * 100 \quad (3)$$

where J_w and J_c are the distilled water flux of the bare and chemically cleaned membrane respectively.

2.6. End-product quality

2.6.1. Total protein analysis

For the experimental samples, the corrected Lowry method was applied for total protein measurement. Various chemicals were used for total protein analysis such as Bovine Serum Albumin (BSA), Copper (II) Sulfate pentahydrate ($CuSO_4.5(H_2O)$), di-Sodium Tartrate ($C_4H_4O_6Na_2.2(H_2O)$), Folin and Ciocalteu's phenol reagent (2N), Sodium Carbonate (Na_2CO_3), and Sodium Hydroxide NaOH. During the total protein analysis by a corrected Lowry method, different chemical solutions were prepared such as Solution A (0.143N of NaOH and 0.135N of Na_2CO_3), solution B (0.057M of $CuSO_4.5(H_2O)$), solution C (0.124M of $Na_2Tartrate.2(H_2O)$) and Folin (dilution ratio is 5 Folin to 6 distilled water). To prepare Lowry solution, solution A: solution B: solution C were mixed freshly with a ratio of 100:1:1 respectively [26]. BSA was used for calibration curve preparation, besides, it was used as a known concentration sample for controlling protein analysis results for other samples. UV/VIS spectrophotometer was operated to measure the absorbance values of the protein by using 660 nm wavelength and consequently protein concentration was calculated.

The rejection rate value of the protein was determined by using the equation below:

$$R\ (\%) = (1 - (C_p/C_f)) * 100 \quad (4)$$

where R is protein rejection (%), C_f and C_p are feed and permeate concentrations (g/L) respectively.

2.6.2. Protein composition analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique was conducted for protein composition analysis purposes by using 16% resolving gel with a 10% (w/v) SDS. To perform SDS-PAGE analysis, the vertical gel electrophoresis unit (Mini-Protein, tetra cell) was used and it was obtained from Bio-Rad Laboratories company. The gels were operated by using a power supply at 100V for 60 min. Coomassie blue stain (10% acetic acid, 0.006% (w/v) Coomassie blue, and 90% H_2O) was used for the staining of protein bands. Isopropanol was used as a fixed solution which was prepared by using 10% acetic acid, 25% isopropanol, and 65% water. Thermo Scientific PageRuler Prestained Protein Ladder was used as a standard for protein types detection.

2.6.3. Lactose analysis

High-performance liquid chromatography (HPLC) was used for lactose analysis. The refractive index detector (RID-10A, SHIMADZU) was used with a BIO-RAD Aminex HPX-87H column (300mm x 7.8mm) and the mobile phase was 0.0065M H_2SO_4. Samples were filtered with 0.45µm filters before using the HPLC.

2.6.4. Antibiotics, hormones, and heavy metals analysis

Antibiotics and hormones analyses were performed by using Liquid Chromatography-Mass Spectrometer (LC-MS/MS) device, Agilent (6460 Model). The analyses were performed according to ASTM D7600-EPA 536 and EPA Method 1694. Heavy metals were measured by using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) device, Perkin Elmer brand, Nexion 300x model. The analysis and calibration for each element were performed according to EPA 6020B method. The samples were taken from feed and concentrate streams. The samples from concentrate streams were taken from the supernatant part after centrifugation for 30 minutes. Samples were filtered with 0.45µm filters before measurement.

3. Results and Discussions

3.1. Performance of disc vs tubular membrane module

3.1.1. Flux analysis

Referring to Figure 3a,b, it was revealed that the changing of membrane module type affected the J/J_w trend of membranes. Using the disc membrane showed a rapid decrease in J/J_w values at both temperatures (40ºC and 50ºC) while the tubular membrane reached the steady-state J/J_w values, immediately. The differences in J/J_w trends of the disc and tubular membranes can be explained by the flow direction of the feed stream along the membrane surface. In the disc membrane module, the feed flow was perpendicular (with tangential movement) on the membrane surface while the feed flow was parallel to the membrane surface in the tubular membrane module. By increasing the whey temperature from 40ºC to 50ºC, J/J_w decreased in the disc membrane module while J/J_w was slightly increased in the tubular membrane module. When the whey temperature increases, the possibility of the attachment of protein molecules to the membrane surface increases and so this affected the overall filterability properties of whey. Pelegrine and Gasparetto [27] reported that when the temperature is between 40°C and 50°C, the protein solubility will be increased. However, at the isoelectric point (IEP) of whey proteins (pH=4.5), the solubility decreased with the temperature due to the effect of the temperature on hydrophobic forces [27]. From the flux graph of 40ºC temperature, as shown in Figure 3a, it can be said that the initial J/J_w values of the disc and tubular membranes were quietly different. At the first 30 min of the filtration experiment, J/J_w value of the disc membrane declines with a percentage of 44% while the declination percentage was 21% with the tubular membrane at the same experiment period. Furthermore, the steady-state of J/J_w for the tubular membrane was higher than the disc membrane at the final stage of filtration experiment. On the other hand, according to the J/J_w graph of 50ºC temperature (Figure 3b), the initial J/J_w values were slightly different between the disc and tubular membrane. Nevertheless, J/J_w was decreasing with a rate of 42% and 14% for disc and tubular membrane respectively after half an hour of filtration experiment. From flux analysis, it can be concluded that the tubular membrane module showed a slow decrease in J/J_w compared to the disc membrane module. The rapid decreasing of J/J_w with the disc membrane leads to the fast fouling due to the perpendicular direction of the feed flow (with tangential movement). Besides, the disc membrane has a more contact surface than the tubular membrane in terms of protein-surface interaction as the filtration area of the disc membrane is larger than the tubular one. This interaction causes high accumulation and aggregation of foulants (e.g. protein) on the surface area. In addition, the tubular membrane modules are operated at the tangential or cross-flow mode in which the feed stream is pumped with high velocities laterally to the membrane surface [24] as the filtration area is less than the disc membrane The high velocity across the tubular membrane surface decreases the contact of foulants (most probably, protein) with the membrane surface and causes more stable flux compared to the disc membrane. These findings confirmed the observation by Ebrahimi et al. [28] as the long-term flux was stable and the fouling was less when a membrane with a smaller area was used. In contrast, the membrane with a larger area showed higher initial flux but it was rapidly decreasing and reached the lowest flux at the long-term experiment.

The differences in membrane filtration performances can be also explained with the pH effect of acidic whey on protein-lactose interaction and so membrane fouling for both module types. In this study, the original pH of whey was held stable near to IEP for observation of the effects of temperature and module type on flux change and end-product quality during acidic whey filtration. The pH of used whey was measured in the range of 4.6-4.8 which is near to the average IEP (4.6) of whey protein [29]. pH values have inconsiderable differences in each stream (feed, concentrate, and permeate). As mentioned by Baldasso et al. [14], it is a good indicator to observe the stability of pH value during the experiment which means that the solution was not degraded. During the initial period of the filtration process, the protein-membrane interaction is the dominant factor for decreasing the flux values and rapid formation of the fouling layer [30]. The protein has minimum solubility at IEP which leads to increase protein-protein interaction which in turn causes protein aggregation and/or precipitation [27]. Konrad et al. [31] determined that the precipitation of whey protein on the membrane surface caused the high membrane resistance/fouling during the concentrating of acidic whey with the UF membrane.

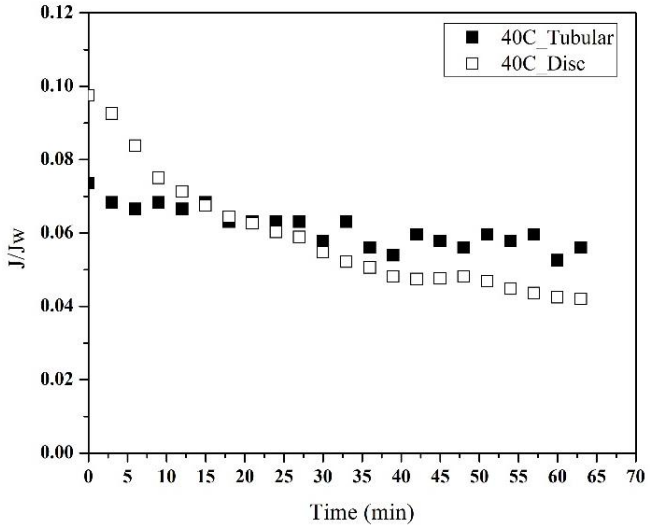
3.1.2. Fouling experiment

During the whey filtration, the fouling is attributed mainly to deposit the whey proteins on the surface of membrane which can cause a decrease in the effective pore size [32]. In the current study, the fouling analysis for different temperatures and membrane module types was investigated with the MFI model. Table 3 illustrates the MFI and α values for different module configurations as well as different feed temperatures. MFI and α values increased from $16x10^6$ to $22x10^6$ and from $2.84x10^8$ m/kg to $4.59x10^8$ m/kg respectively with the increase of temperature from 40ºC to 50ºC with the disc membrane which is in line with J/J_w results. MFI is correlating linearly with the particle concentration in the feed [33] and a higher value of α and MFI

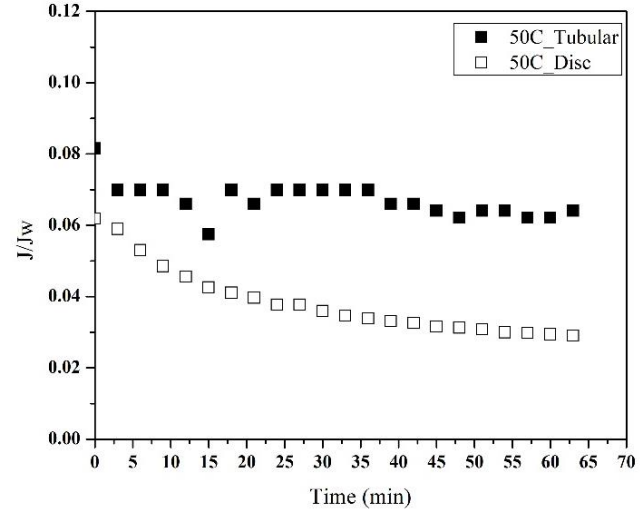
indicate high membrane fouling. In a tubular membrane module, MFI values were determined as $8x10^6$ and $4x10^6$ at 40ºC and 50ºC respectively. While α values were calculated as $1.28x10^8$ m/kg at 40ºC and $1.08x10^8$ m/kg at 50ºC.

These values showed that the lower fouling was achieved by using the tubular membrane module at both temperatures compared to the disc membrane module. In addition to that, increasing the temperature by more than 40ºC may lead to denaturation of protein which can decrease the permeate flux and increase the membrane fouling because of the protein precipitation and aggregation on the membrane surface [19,20]. This precipitation and aggregation are likely to take place on the disc membrane surface rather than the tubular one as the feed flow direction is moving perpendicularly on the surface of the disc membrane.

After applying chemical cleaning on the fouled ceramic membranes, the flux recovery rate of the used membranes was calculated. Referring to Figure 4, it was observed that for both disc and tubular membrane, the flux of distilled water after chemical cleaning was almost the same as the flux of distilled water for the bare membrane. Consequently, the fouled membranes could be successfully recovered with a percentage of up to 98%. It showed that even when more fouling happened with the disc membrane and at a higher temperature, the membranes could be recovered successfully with the chemical cleaning.



(a)



(b)

Fig. 3. J/Jw of whey filtration for tubular and disc membrane modules at (a) 40ºC, and (b) 50ºC.

3.2. End-product quality

The separation performance of protein and lactose with increasing temperature has great importance for industrial applications due to the production of high protein products at hot feed because of the hygienic aspect. The optimum operating condition should give high content of protein and low content of lactose in the concentrate stream of the UF membrane process for high end-product quality. At the ceramic membrane operations, the temperature can efficiently change the fractionation of whey protein. Besides, the module configuration contributes to the separation of protein and lactose in cheese whey. In terms of hygienic aspects, antibiotics, hormones, and heavy metals should be controlled in the end-products due to their negative impacts on human health.

3.2.1. Total protein

Figure 5 shows the protein rejection and concentration values in the disc and tubular membrane module at whey temperatures of 40ºC and 50ºC. The protein rejection on disc membrane was slightly more than the tubular membrane as the protein concentration in the permeate of the disc membrane is less than the tubular membrane. This can be explained that the differences in the feed flow direction between both membranes may change the capture of protein molecules by membrane contact zone. It means that more protein molecules are captured by the membrane surface in the disc membrane module than the tubular membrane module. Besides, the higher filtration area of the disc membrane and higher initial flux especially at 40ºC led to accumulating more protein and so higher rejection. Similar to Ebrahimi et al. [28] study, they realized that the rejection on the membrane surface was lower when the initial flux was less. In the current study, the protein rejection on the tubular membrane was calculated as 89% and 87% at 40ºC and 50ºC respectively, while the protein rejection was increased to 94% and 96% at 40ºC and 50ºC respectively by using the disc membrane module.

As shown in Figure 5, the protein concentrations were measured in the concentrate part as 6.9 g/L and 4.1 g/L for the tubular membrane and 6.1 g/L and 6.9 g/L for the disc membrane at 40ºC and 50ºC respectively. The concentration increment was calculated as a ratio of protein concentration between feed and concentrate stream. For tubular membrane, protein increment was found as 1.7 at 40ºC and 1.2 at 50ºC which means that more protein could be recovered when the whey temperature was 40ºC. For disc membrane, protein concentration had been doubled in the concentrate stream at both 40ºC and 50ºC. This shows that the temperature of cheese whey did not affect significantly the recovered protein from the disc membrane. However, higher protein had been recovered from the tubular membrane when the temperature of cheese whey was lower. This can be explained as the tubular membrane had a higher flux rate with a higher temperature which leads to less protein to be retained in the concentrate stream when the temperature is higher.

Table 3
MFI and α values for different temperatures with different module configurations.

Temperature (°C)	Disc Module		Tubular Module	
	MFI	α (m/kg)	MFI	α (m/kg)
40	$16*10^6$	$2.84*10^8$	$8*10^6$	$1.28*10^8$
50	$22*10^6$	$4.59*10^8$	$4*10^6$	$1.08*10^8$

It was expected that the protein is prone to aggregate or precipitate with increasing temperature. Donato et al. [34] explained these phenomena with microscopic observations of the whey protein aggregation mechanism that the increasing temperature (from 70ºC to 85ºC) caused to increase Z-average hydrodynamic diameter (D_h). Furthermore, the microscopic observations showed that the shape of particles changed as spherical or elongated depending on heating conditions. These changes of shape and size in protein molecules probably affect the interaction between lactose and protein and so their membrane separation mechanism.

3.2.2. Protein composition

Figure 6 shows the whey protein types from SDS-PAGE analyses that are available in the feed and concentrate stream of acidic cheese whey at different temperatures with tubular and disc membrane. α-La, β-Lg, Casein, and BSA are the protein types that were found in both tubular and disc membrane modules at different temperatures. Combined β-Lg and α-La were observed in the tubular membrane module while they were fractionated with the disc membrane module. The dominant type of protein was β-Lg during the experiment with the disc membrane module. For a purpose of whey protein fractionation, the disc membrane module would be a promising suggestion. In Prabhuzantye et al. [35] study, they found conspicuously three types of protein (β-Lg, α-La, and BSA) in the liquid raw whey, liquid retentate whey, and dried whey protein concentrate. In another study by Nicolás et al. [36] they observed Lactoferrin type in addition to β-Lg, α-La, and BSA in the untreated whey.

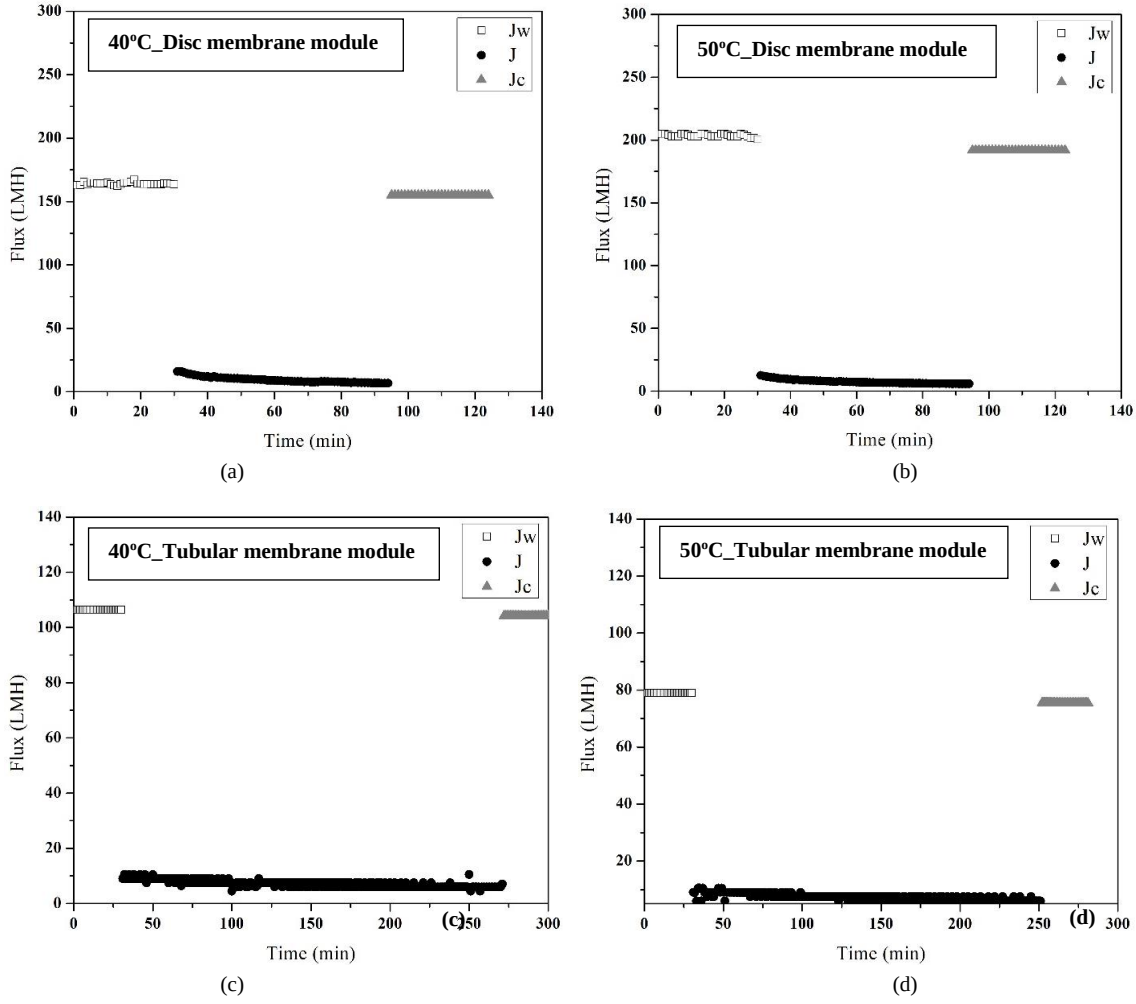


Fig. 4. The permeate flux of distilled water for the bare member (Jw), whey (J), and distilled water after chemical cleaning (Jc) for different temperatures and module configurations.

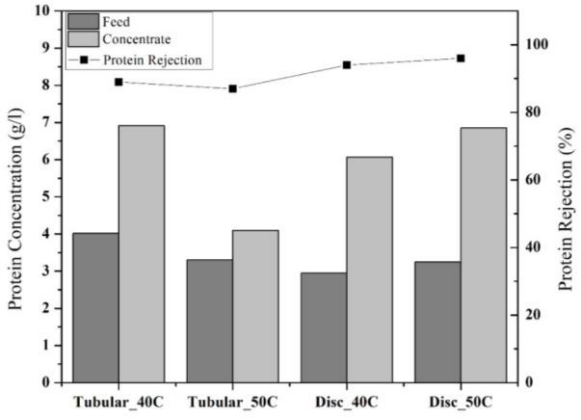


Fig. 5. Concentration and rejection percentage of protein at 40ºC and 50ºC for tubular and disc membrane module.

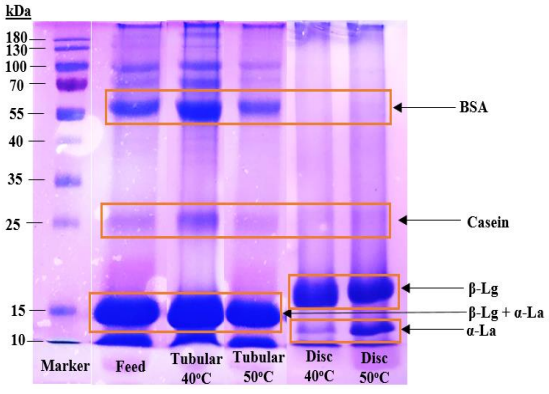


Fig. 6. SDS-PAGE analysis for different temperatures with tubular and disc membrane module

3.2.3. Lactose

Figure 7 shows the lactose concentrations in both disc and tubular membrane modules at different operation temperatures. The lactose concentrations were measured in the concentrate part as 9.7 g/L and 16.1 g/L for the tubular membrane and 45.8 g/L and 49.7 g/L for the disc membrane at 40ºC and 50ºC, respectively. However, the lactose concentration in the feed used for the tubular membrane was lower than the feed used for the disc membrane. The increasing percentage of lactose between feed and concentrate stream was calculated for both membranes at both feed temperatures. It was found that the lactose was increasing with an average percentage of 3% at 40ºC and 10.5% at 50ºC for both tubular and disc membrane. It was concluded that the module configuration did not affect significantly the end-product quality of the cheese whey in terms of lactose content. In contrast, the temperature of whey showed a substantial effect on the lactose content in the cheese whey. This can be explained as higher temperature applied, higher interaction has occurred between lactose and protein which leads to higher accumulation of the lactose in the concentrate stream. This result is not preferable in terms of end-product quality, as the concentrate stream should contain as fewer as possible of lactose content, so pure protein could be recovered.

3.2.4. Antibiotics, hormones, and heavy metals

Amoxicillin and erythromycin were investigated as possible types of antibiotics that can be presented in the end-product of the cheese whey. It was detected that the concentration of amoxicillin in the concentrated part was reduced to half comparing to its concentration in the feed. On the other hand, erythromycin concentration in the concentrate stream was increased by more than three folds of its concentration in the feed. The admissible amount of erythromycin in milk was mentioned to be around 40 µg/kg (i.e. 44.5 ppb) [37] and 60% of the erythromycin is transferred to the whey [8]. According to that, the admissible amount of erythromycin in whey is estimated to be 24 µg/kg (i.e. 26.7 ppb). By comparing the erythromycin results of this study to the admissible amount, it was noticed that the maximum erythromycin concentration in the concentrated part (3.5 ppb) was much lower than the admissible amount of erythromycin in the whey (26.7 ppb). In terms of amoxicillin, the recommended maximum residue limit in livestock milk is 4 µg/kg (i.e. 4.45 ppb) [38] and 100% of the amoxicillin is transferred to the whey which means almost all of the amoxicillin is released from cheese curd during the draining process [8]. By comparing the amoxicillin results of this study to the maximum residue limits, it was detected that the maximum amoxicillin concentration in the concentrate stream (0.1 ppb) was much lower than the maximum residue limits of amoxicillin in the whey (4.45 ppb). The high transfer percentage of amoxicillin and erythromycin from milk to cheese whey is due to their high solubility nature [8].

Estradiol (E2) was investigated in the feed and concentrate stream as a possible type of hormone that can be presented in the end-product of the cheese whey. It was observed that the concentration of E2 was less than 0.05 ppb in all samples which indicates that the source of milk had a minimum amount of E2. Wolford and Argoudelis [39] found a very low amount of E2 in the whey with a concentration of 1.5±0.2 pg/mL which is equal to 0.0015 ppb. In addition, they found that amount of E2 in the whey was the minimum compared to Estrone (E1) and Estriol (E3).

Table 4 below shows the heavy metal concentrations in the feed and concentrate stream of the cheese whey used in this experiment as well as the concentration in raw whey from the literature. It was observed that almost all of the heavy metal amounts in this study were less than the concentrations found from different studies in the literature. Most of the heavy metals had a higher concentration in the concentrate stream except chromium and selenium. Some heavy metals were significantly reduced in the concentrate stream compared to their concentration in the feed. Chromium is an example of that reduction when the concentration decreased from 16.79 ppb to 1.7 ppb with a reduction percentage of around 90% which means that most of the chromium amount passed through the permeate stream. On the other hand, some of the heavy metals were significantly increased in the concentration stream. Cadmium and iron were increased by 3.3 and 1.6 folds respectively regarding their content in the feed. Also, it was observed that boron content was the maximum between all heavy metals in addition to aluminum and zinc which showed a relatively high concentration in both feed and concentrate.

Generally, trace elements or heavy metals are present in different food products in acceptable concentrations to meet the body requirement. Besides, the taken dosage of those trace elements should be limited so it will not affect negatively human health. However, the dosages are tolerating between adults and children as their needs for the trace elements are different. The provisional tolerable weekly intake (PTWI) mg/kg body weight of different heavy metals for adults are 0.015, 0.007, 0.025, and 0.0016 mg/kg body weight for arsenic, cadmium, lead, and mercury respectively according to Fact Sheet No. 4.4 published by World Health Organization (WHO) [40].

Moreover, according to European Commission (EC) No 1881/2006 [46], the maximum level of cadmium in liquid and powder infant formula is 0.005 and 0.01 mg/kg respectively and in the range of 0.04-3.0 mg/kg in other foodstuffs. By comparing the results of this study, the concentration of cadmium in both feed and concentrate was found as $4.8x10^{-5}$ mg/kg which much lower than the maximum acceptable limit. In terms of arsenic, the maximum level in different foodstuffs is ranging from 0.1 to 0.3 mg/kg according to the European Commission [46]. The current study showed that the amount of arsenic in the feed and concentrate stream was 0.006 and 0.007 mg/kg respectively which is much lower than the maximum limit.

Trace elements and heavy metals are also present in animal feed and their maximum limits are also regulated according to European Commission (EC), 2002/32. The maximum content of arsenic and cadmium is 2 ppm and 1-2 ppm respectively in feed materials and complete feeding stuff [47]. By comparing the results of this study to European Commission regulation, it was found that both arsenic (0.0011 ppm) and cadmium (0.00004 ppm) in feed were much lower than the maximum limit. Moreover, although arsenic and cadmium concentrations in the concentrate stream were more than the concentrations in feed, their concentrations still much lower than the maximum limit of the European Commission (EC).

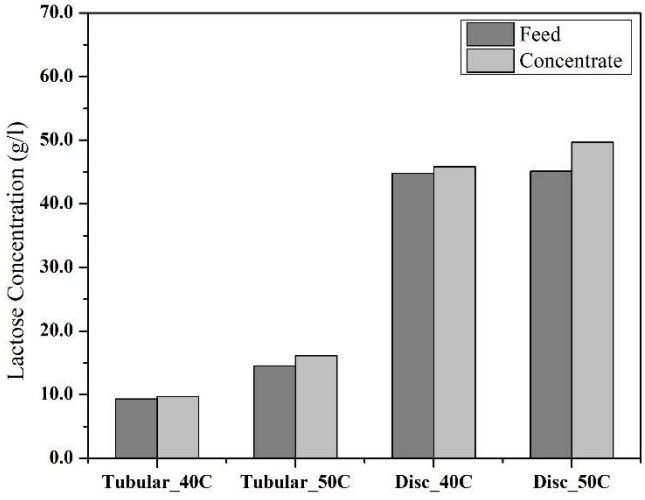


Fig. 7. Lactose concentration in feed and concentrate stream at 40ºC and 50ºC for tubular and disc membrane module.

4. Conclusions

Various findings were obtained from this study in terms of J/J_w, fouling mechanism, and end-product quality by applying different feed temperatures and by using different module configurations. It was concluded that the increase of temperature may cause the structural changes of whey molecules and will affect the performance of whey filtration. Additionally, tubular membrane modules revealed a high ability to treat solutions with high level of suspended solids without plugging the membrane even with higher temperatures. The high cross-flow velocity of feed solution helps to achieve stable and high flux as well as the simplicity during the membrane cleaning by mitigating the improvement of a concentration polarization layer across the membrane surface. In terms of separation efficiency, the whey temperature plays a significant role in the end-product quality rather than module configuration. The protein could be recovered in a good manner when a lower temperature was applied (40ºC) especially in the tubular membrane module. Nevertheless, the accumulation of lactose in the concentrate stream was less at a lower temperature which leads to better purification of the protein as lower lactose is present. On the other hand, β-Lg, α-La, BSA, and Casein are the main protein types that were observed in the cheese whey with both modules.

Tubular or disc membrane can be suggested to be used depending on the specific application purposes. For obtaining higher protein, the disc

membrane would be suggested according to the high accumulation of protein in the concentrate stream. However, membrane fouling should be controlled frequently. On the other hand, when lower fouling operation is needed for long-term applications, tubular membranes would be offered according to their low membrane fouling index even with higher temperatures. In addition, the end-product quality in terms of the content of protein and lactose is more or less comparable with the quality from the disc membranes. Regarding the end-product quality of antibiotics, hormones, and heavy metals, it was concluded that they did not have a negative impact on human health as their concentrations were less than the permissible limits in the end-products of cheese whey.

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List of abbreviations

α	Specific cake resistance
α-La	α-Lactalbumin
β-Lg	β-Lactoglobulin
BSA	Bovine serum albumin
HPLC	High-Performance Liquid Chromatography
IEP	Isoelectric point
J	Whey permeate flux
Jc	Distilled water flux after chemical cleaning
Jw	Distilled water flux of the bare membrane
J/Jw	Normalized flux
kDa	Kilo Dalton
MF	Microfiltration
MFI	Membrane Fouling Index
MWCO	Molecular Weight Cut-Off
NF	Nanofiltration
RO	Reverse osmosis
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
UF	Ultrafiltration

Table 4
Heavy metal concentrations of cheese whey in feed and concentrate stream.

Heavy metals	Concentration (ppb) (Feed)	Concentration (ppb) (Concentrate)	Value (mg/kg) (Feed)	Value (mg/kg) (Concentrate)	Concentration in Whey (Literature) (mg/kg)	Reference
Aluminum (Al)	71.64	100.09	0.37	0.34	7.085*	[41]
Arsenic (As)	1.1	1.23	0.006	0.007	0.52 0.01*	[42] [41]
Boron (B)	118.29	127.23	0.563	0.539	-	-
Cadmium (Cd)	0.04	0.13	<0.048 µg/kg	<0.048 µg/kg	0.02-3.7µg/kg 9.0 µg/kg *	[43] [41]
Chromium (Cr)	16.79	1.7	0.058	0.007	0.02	[42]
Iron (Fe)	34.81	54.23	Negative	Negative	2.5 - 3.4 1.06 0.3	[43] [44] [45]
Selenium (Se)	0.69	Negative	0.175	Negative	n.d. * 4.56 µg/kg	[41] [45]
Zinc (Zn)	60.99	72.40	0.10	0.23	1.2 - 2.2 0.23 2.34 1.2	[43] [42] [44] [45]

* In whey powder
n.d.: not detected

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