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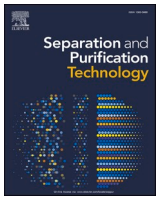

Impact of feed properties on membrane performance for ultrafiltration of charged solutes

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ABSTRACT

Four polyethersulfones spiral wound ultrafiltration (UF) membranes with a nominal molecular weight cut-off (MWCO) of 5 kDa and 1 kDa were applied to separate neutral and charged solutes. The effects of the charge properties of solutes on membrane performance (permeate flux and retention) were evaluated. The membranes with the same nominal MWCO did not always provide similar membrane performance for uncharged and charged solutes. Similar permeate fluxes did not correspond to similar membrane retention. In the case of similar nominal MWCO, a higher membrane permeability likely resulted in increasing membrane retention. The charge states of solutes did not influence the permeate flux, but an increase in feed concentration did significantly decrease the flux. Therefore, membrane retention depends on the charge properties of solutes and the feed concentration. The UF membranes were used to fractionate an industrial fish protein hydrolysate at a high concentration. Although the adjustment of the pH and ionic strength changed the charge properties of peptides in the hydrolysate, the charge interactions did not significantly impact the membrane performance. The four membranes produced permeate products with different molecular weight distributions. Despite these differences, the products still had the same amino acid profile. The retention factors of amino acids were slightly dependent on their hydration numbers, with more hydration giving slightly lower retention. This means that the UF of fish protein hydrolysate was mainly based on size exclusion. In general, the membranes retained more peptides containing leucine, phenylalanine and methionine.

1. Introduction

Ultrafiltration (UF) is a pressure-driven membrane separation technique. It is based on size exclusion, meaning that molecules bigger than the membrane pores are retained by the membrane, and the smaller molecules permeate through the pores. UF separation is classified as a mild separation process, as it does not require high temperatures or extreme pressures, consequently preventing the deterioration of food nutrients [1,2]. This technique is also energy efficient and does not require much auxiliary chemicals compared to other separation processes, such as distillation, chromatography, and chemical extraction. UF is widely used in the food industry because of the abovementioned efficiency and sustainability. Applications are mainly for concentration and clarification purposes, e.g. concentrating whey proteins and clarifying juices [2–5]. However, there are still limitations [2]. Fouling and membrane pore blocking are serious problems when using UF membranes for the separation of macromolecules, particularly for separating proteins. Another problem is the relatively low selectivity, especially when compared to chromatography techniques. These limitations can be overcome with proper process design and the optimization of operating conditions [1,2]. Insight into separation mechanisms is needed to accomplish both systematic design and optimization.

A recent application of UF membranes is the fractionation of peptides from protein hydrolysates [6–12]. Native proteins are hydrolyzed into short-chain peptides containing different amino acid residues with unique molecular properties, such as size, charge, hydrophobicity, hydration, etc. By this conversion, peptides with specific bioactivities can also be released. Some protein fragments show specific bioactivities, such as antioxidant, antihypertensive, and Angiotensin-I-Converting Enzyme (ACE) inhibition activity [13–15], that are useful for nutraceutical and pharmaceutical applications. However, not all peptides obtained from hydrolysis contain the desired bioactivities. To enhance the properties, downstream processing is needed to separate targeted peptides [12]. Several studies reported that bioactive peptides are usually smaller than 4,000 Da [16–18] and mostly contain hydrophobic amino acid residues [8,19–22]. Given these observations, ultrafiltration,

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defined as membranes having a nominal molecular weight cut-off of 1,000 – 500,000 Da and applying pressure below 10 bar [23,24], is a potential separation technique that can be used to fractionate peptides with desired bioactive properties.

Applying UF to separate bioactive peptides smaller than 4,000 Da seems simple and straightforward. However, the separation of charged molecules is not based only on size exclusion but also on other interactions that could influence the separation. Those interactions are caused by the unique properties (charge, hydrophobicity, and hydration) of molecules in a solution and specific membrane characteristics. For a semi-permeable UF membrane, solvent and solute molecules that are smaller than membrane pores can permeate through them, whereas larger solutes are rejected and accumulate at the membrane surface because of the membrane retention. The accumulation creates a layer before the membrane surface, which is known as a concentration polarization (CP) layer. These accumulated solutes diffuse back to the bulk solution owing to the difference in concentrations, producing a concentration gradient along the CP layer [25,26]. The interactions between the membrane surface and the retained molecules determine the properties of the layer. Within the CP layer, repulsive and attractive forces between peptide molecules result in molecular association and dissociation. Charge interactions can significantly impact the separation during UF separations for proteins [27–33] and protein hydrolysates [34–43]. Other interactions originate from differences in hydrophobicity [39,44]. Molecular properties, such as solubility and hydration, have not been considered yet.

Until now, simple mixtures with often single or a few proteins or amino acids were used as the model systems to prove the effects of charge and hydrophobic interactions on separation mechanisms during UF separations. The study of simple mixtures provides insight into individual molecular interactions and their effects on separation. It is, however, practically challenging to translate these results towards protein hydrolysates containing many different peptides and free amino acids, as many emergent properties in complex mixtures are not yet included. While the simultaneous effects of all interactions are indeed very complex, they still originate from unique molecular properties and interactions. Therefore, studying a complex mixture, and relating the performance to the properties of the constituents is a good approach to practically evaluating those interactions. The charge and hydrophobic interactions of peptides in dairy protein hydrolysates have been intensively studied for decades. Dairy hydrolysates generally contain less than 30 main peptides [38,39,41,42,44–47] since dairy proteins are not as complex as muscle proteins. In the case of protein hydrolysates obtained from muscle proteins like fish, the hydrolysates contain thousands of peptides with different properties. This suggests that more interactions take place in front of the membrane, which could severely affect the separation.

In previous research [43], we studied the UF fractionation of a fish protein hydrolysate (Prolastin) at a low concentration of 0.5 % (w/v). The change in the environment (pH and ionic strength) modified the membrane performance and selectivity. In addition to size exclusion, the overall charge state of the peptides was indicated as a dominant interaction that affected separation. The current study aims to clarify the effect of charge interactions on UF-based size separation. For this reason, we investigated the impact of charged solutes on membrane performance. This was done with fish protein hydrolysate at an elevated concentration of 5 % (w/v), which is comparable to the actual concentration of a commercial fish protein hydrolysate after enzymatic hydrolysis [48]. In addition, the membranes were characterized with uncharged dextrans and maltodextrin. As aforementioned, unique molecular properties of solutes could affect the separation. Therefore, the amino acid profiles of the peptide fractions were analyzed to determine the relationship between molecular properties and solute retention.

2. Materials and methods

2.1. Materials and chemicals

Dextran 6 kDa from *Leuconostoc* spp. with a molecular weight of 4,500 – 7,500 Da and maltodextrin with dextrose equivalent 4.0 – 7.0 were purchased from Sigma-Aldrich (Steinheim, Germany). Prolastin, a commercial fish protein hydrolysate, was from Copalis (Le Portel, France). The hydrolysate was obtained from the enzymatic hydrolysis of marine elastin. The hydrolysate material contains low salt and high protein contents of 0.73 – 0.79 % (w/w) and 88.5 % (w/w), respectively [49,50]. The dextran, maltodextrin and Prolastin were characterized by the HP-SEC technique (*Section 2.4.2*) and the molecular weight profiles are given in Figs. 1 and 2.

Sodium hydroxide (NaOH) for analysis was from Merck (Germany). Hydrochloric acid 37 % (HCl) and sodium azide were purchased from VWR Chemicals (France). Acetonitrile Ultra LC-MS and methanol HPLC gradient were of analytical grade from Actu-All Chemicals (Netherlands). Trifluoroacetic acid with a purity of 95 % was purchased from Thermo Fisher Scientific (France). Phenol, disodium phosphate, sodium tetraborate and sodium chloride (NaCl) with a purity of $\geq$ 99.5 % were from Sigma-Aldrich (Germany).

2.2. Spiral wound ultrafiltration membranes

Four spiral wound membranes were selected from Sterlitech (US). All membranes are categorized as polyethersulfone (PES) membranes. Two membranes were manufactured by Synder Filtration (US) with a nominal molecular weight cut-off (MWCO) of 5 kDa (MT series) and 1 kDa (XT series). The other membranes were from Suez (Lenntech, US) with a nominal MWCO of 5 kDa (PT series) and from MANN + HUMMEL (MICRODYN-NADIR, Germany) with a nominal MWCO of 1 kDa (NP010 series). Table 1. shows the specifications of all membranes.

All membranes were cleaned before and after use with commercial cleaning agents from Sealed Air, Diversey Care (Netherlands). The cleaning protocol recommended by Diversey consists of three steps: enzymatic, acidic, and alkaline cleaning. The enzymatic cleaning was done by recirculating a mixture of Divos 90 (1 % w/v) and Divos 80–2 (0.35 % w/v) for 40 min. After that, acidic cleaning was started by recirculating Divos 2 (0.5 % w/v) for 20 min. The last alkaline cleaning with Divos 116 (0.6 % w/v) was run for 30 min. All cleaning steps operated at 50 °C. Unused membranes were stored in Divos LS (0.6 % w/v) adjusted to a pH of 2 – 3.

2.3. Filtration experiments

2.3.1. Preparation of feed solution

Dextran 6 kDa and maltodextrin DE 4.0 – 7.0 with a concentration of 3.5 and 1.5 g/L, respectively, were both dissolved in MilliQ water to make a 0.5 % (w/v) solution. A 5 % (w/v) solution of Prolastin was prepared and adjusted to pH 5 or 8 with 2 M NaOH or 2 M HCl. For solutions with elevated ionic strength, 0.6 M NaCl was added to the Prolastin solution. All solutions were pre-filtrated by Whatman glass microfiber filters, Grade GF/D, to remove aggregates, clumps and dust from the solutions, and used within 24 h.

2.3.2. Filtration experiment

All filtration experiments were performed using a pilot-scale system. The configuration of the system was previously described [53,54]. Before starting an experiment, the membrane permeability was measured using demineralized water at a cross-flow velocity of 0.1 m/s and at 25 $\pm$ 0.5 °C. The water flux was quantified at 2, 4 and 6 bar. After this water measurement, a filtration experiment was done by fractioning five liters of feed solution. The fractionation was operated at the same velocity, temperature and pressure as set for the water test. The experiment was conducted in recirculation mode by recycling both retentate

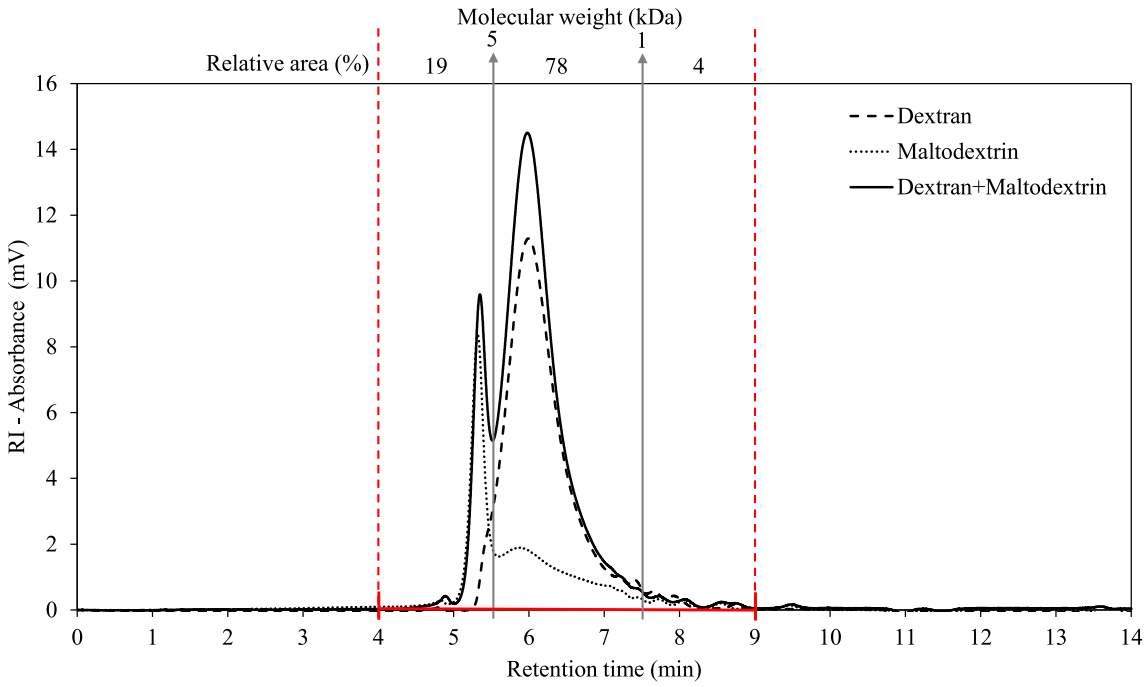

Fig. 1. Molecular weight distribution of 0.34 % (w/v) dextran 6 kDa, 0.14 % (w/v) maltodextrin DE4-7 and the mixture of dextran and maltodextrin with a concentration of 0.5 % (w/v). The relative area belongs to the chromatogram of the mixture. (three replications; standard deviations of relative area were ± 1).

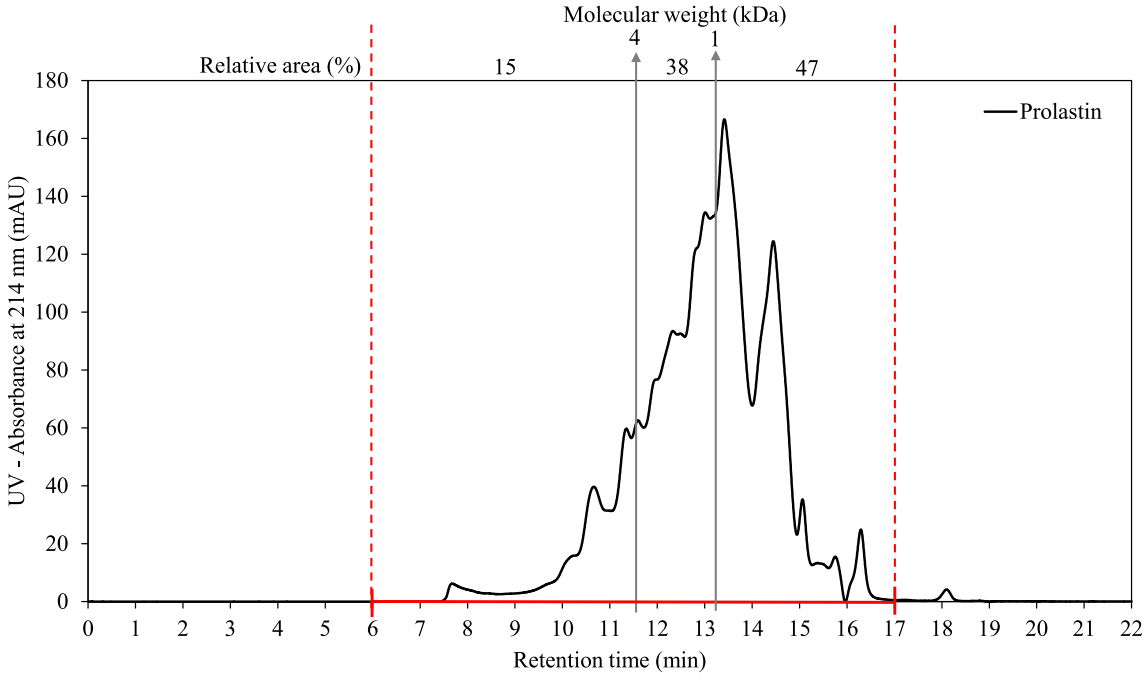

Fig. 2. Molecular weight distribution of 0.5 % (w/v) Prolastin with the relative area of peptide fractions. (three replications; standard deviations of relative area were ± 1).

and permeate streams back to the feed tank. All set parameters and permeate flow rate were automatically measured and recorded, except the flow rate when using the 1 kDa membranes. This was manually measured for 5 min before sample collection. After the permeate flow rate reached a constant value, usually within 45 min, samples from the retentate and permeate streams were taken for analysis. Most experiments were done in duplicate.

2.3.3. Reversibility test

The filtration condition was at 25 ± 0.5 °C with a cross-flow velocity of 0.1 m/s (the maximum limitation for the machine). The reversibility test began with evaluating the membrane permeability of the cleaned membrane. After that the membrane was used to filtrate the hydrolysate at 2, 4 and 6 bar; then the pressure was reversed back to 4 and 2 bar again. The filtration time was 45 min for each pressure. After the filtration, the membrane system was flushed with demi-water with a cross-flow velocity of 0.1 m/s. Then, the permeability was tested again.

Table 1
Characteristics and nominal molecular weight cut-off specification (by supplier) of the membranes.

Membrane	MT series	PT series	XT series	NP010 series
Manufacturer	Synder	Suez	Synder	NADIR
Material	PES	PES	PES	PES
pH Stability range[a]	2–10	2–10	2–10	0–14
Maximum temperature(°C)	55	50	55	50
Maximum inlet pressure(bar)	8.3	13.7	8.3	40
Nominal MWCO (kDa)	5	5	1	1
Effective area(m^2)	0.3344	0.3700	0.3344	0.3700
Zeta potential(mV)	−14.7 ± 1.6 [51]	N/A	negative[b]	−12.4 at pH 7;−15 at pH 11 [52]

[a] at ambient temperature.
[b] assumed to be negative as PES membranes from Synder have negative zeta potential values [51].

After that, chemicals were applied to clean membrane and system; then, the membrane permeability was evaluated.

The (membrane) permeability was calculated based on Equation (2) (see *Section 2.4.1*). The resistance or the reciprocal of permeability was also estimated.

2.4. Analyses

2.4.1. Volumetric permeate flux and permeability

The flux (J_v, L/h·m^2) is the ratio of the permeate flow rate (q_p, L/h) and the membrane area (A, m^2) as shown [55]:

$$J_v = \frac{q_p}{A} \tag{1}$$

Additionally, the permeability of a membrane (L_p, L/m^2·h·bar) can be estimated based on Darcy's and Hagen-Poiseuille's laws by

$$L_p = \frac{J_v}{\Delta p} \tag{2}$$

where Δp is the transmembrane pressure.

2.4.2. Molecular weight distribution (HPLC analysis)

The molecular weight distributions of dextran 6 kDa and maltodextrin DE 4.0 – 7.0 were characterized by high-performance liquid chromatography. A Shodex column (KS-802 8.0 × 300 mm, Japan) was applied to separate solutes by size. A refractive index detector (Shodex RI-501, Japan) was used to detect the solute concentrations. The column was operated at 50 °C with MilliQ water as eluent at a flow rate of 1 mL/min. The retention times used to characterize molecular weights are presented in Table S1 (supplementary material, appendix S1).

All Prolastin samples were analyzed by HP-SEC using consecutive TSKgel G3000SWxl and TSK gel G2000SWxl columns from Tosoh Bioscience GmbH (Germany) to characterize their molecular weight distributions. The mobile phase was 30 % (v/v) acetonitrile and 0.1 % (v/v) trifluoroacetic acid. The analysis was operated at 30 °C with a flow rate of 1.5 mL/min. UV absorption was detected at 214 nm. The retention times and molecular weights for the analysis were summarized in Table S2 (supplementary material, appendix S1). All samples were diluted ten times to reduce interference of high salt content.

2.4.3. Retention factor

The retention factor (R, −) reflects the ability of a membrane to retain solutes in a solution. The retention factor can be calculated by [56]

$$R = 1 - \frac{c_p}{c_r} \tag{3}$$

where c_p and c_r are the concentrations of a desired component in permeate and retentate samples, respectively. According to Vandanjon et al. [57], the retention factor can also be determined with

$$R = 1 - \frac{\mathrm{AUC}_p}{\mathrm{AUC}_r} \tag{4}$$

where AUC_p and AUC_r are the total surface areas under the HP-SEC chromatograms of a desired component for the permeate and retentate samples, respectively. This is valid as long as the solute concentration is linearly correlated with the surface area under the chromatogram ($c_i \propto AUC_i$). The retention factor of each peptide fraction (R_i) was also estimated using the same assumption:

$$R_i = 1 - \frac{\mathrm{AUC}_{p,i}}{\mathrm{AUC}_{r,i}} \tag{5}$$

2.4.3.1. Retention profile. The molecular weight distributions of the retentate and permeate samples were used to calculated 'retention at a time' (R_t)

$$R_t = 1 - \frac{\mathrm{Absorbance}_{p,t}}{\mathrm{Absorbance}_{r,t}} \tag{6}$$

Equation (6) is valid under the assumption of the linear correlation between solute concentration and absorbance intensity (Beer-Lambert law). We also assumed that the retention times for HP-SEC analysis are identical for all samples. The retention at a time was plotted against retention times to create a retention profile. The retention times were related to the molecular weight of solutes as shown in Table S1 and S2 (supplementary material, appendix S1) for neutral and charged solutes, respectively.

2.4.3.2. Average retention factor. The retention factors calculated from Equation (4) of each membrane were averaged regardless of varying pH, salt content and transmembrane pressure, as follows:

$$R_{average} = \frac{\sum_{j=1}^{j=n} R_j}{n} \tag{7}$$

where n is the number of experimental conditions.

2.4.4. Titration analysis

Prolastin samples were titrated using a 877 Titrino plus auto-titrator (Metrohm, Netherlands). A sample was diluted ten times before titrating. The diluted sample was titrated with 0.3 M HCl at a rate of 0.1 mL/30 s until the solution pH reached pH 2 and was then titrated back by adding 0.3 M NaOH until pH 11. The amount of HCl and NaOH from the titration was used to calculate the differential buffering capacity (dB/dpH) of each sample:

$$\frac{\mathrm{dB}}{\mathrm{dpH}} = \frac{\text{amount of HCl or NaOH added to reach the desired pH}}{\text{pH change} \times 50\ \text{mL solution} \times \text{peptide concentration in sample}} \tag{8}$$

After that, the dB/dpH obtained was plotted versus pH from 2 to 11 to make a buffering curve/profile.

2.4.5. Pretreatment for determination of amino acid profile

The amino acid analysis consists of analysis of both free amino acids and hydrolyzed samples.

2.4.5.1. Free amino acid. The solutions of 400 µL methanol and 100 µL norvaline (0.4 mM) were added to 500 µL diluted sample and then mixed to precipitate peptides/proteins in the sample. After that,

centrifugation was done at 9000 rpm for 10 min using a centrifuge (5424, Eppendorf, Germany). The supernatant part was filtrated by a 0.2 µm filter before putting into an analysis vial.

2.4.5.2. Hydrolyzed sample. A diluted sample of 100 µL was put into an analysis vial and dried under vacuum using the Eldex Hydrolysis/Derivatization WorkStation (H/D WorkStation), Eldex, US. The dried sample was added to a reaction vial containing excess 6 N HCl with 1 % (w/v) phenol. The vapor phase hydrolysis was taken place at 110 °C for 24 h under a vacuum state in the oven of the H/D WorkStation. After the hydrolysis, the hydrolyzed sample was dried under vacuum and kept at room temperature in the dark before U-HPLC analysis. Table S3. (supplementary material, appendix S1) shows the losses of amino acids from the acid hydrolysis. The hydrolyzed sample was added by a solution of 800 µL methanol and 200 µL norvaline (0.4 mM) and further treated as described in *Section 2.4.5.1*. A 0.5 mL filtered sample and 0.5 mL MilliQ water were put and mixed in an analysis vial.

2.4.6. Determination of amino acid profile

All prepared samples were analyzed using ultra-high performance liquid chromatography (U-HPLC, Thermo Fisher (Dionex) Ultimate 3000) using Acquity UPLC BEH C18 1.7 µm, 2.1x150 mm column from Waters Corporation (Massachusetts, US), operated at 50 °C. Eluent A was a mixture of 10 mM disodium phosphate, 10 mM sodium tetraborate and 2 mM sodium azide, adjusted pH to 7.8 using concentrated HCl. Eluent B was Methanol-Acetonitrile and MilliQ water in the ratio of 20–60-20. The flow gradient of the eluent solutions is shown in Table 2. The amino acid concentrations were quantified by UV adsorption at 338 nm and 263 nm. The standard curve for the analysis was made from Amino Acid standard AAS18 (Merck, Germany) and Asparagine, Glutamine, Tryptophan, Ornithine and GABA (2.5 mM) from Sigma-Aldrich (Germany). All hydrolysate samples were done in duplicate and diluted five times with MilliQ water, except the permeate samples obtained from the 1 kDa membranes. Those permeate samples were diluted by 2 times.

2.4.7. Calculation of hydration number

The hydration number of individual amino acids was estimated based on the study of Burakowski and Gliński [58]. The hydration number of an amino acid was the summation of the hydration numbers from its functional groups at a specific solution pH (Table S4-S5., supplementary material, appendix S1).

2.4.8. Statistical analysis

The average water permeability values of all membranes were statistically compared by performing analyses of variance (ANOVA), followed by using Tukey's HSD (honestly significant difference) test to identify the significant difference between those values. The average retention factors of all experiments were also tested by ANOVA. Tukey's HSD test was applied to the retention data to identify the statistical significance of the difference between the retention factors obtained from different membranes used to filtrate solutions containing neutral and charged solutes. RStudio software (version 4.4.1) was used for the statistical analysis.

Table 2
Flow gradient of the eluent A and B for amino acid analysis.

Time (min)	Flow rate (mL/min)	Eluent A (%)	Eluent B (%)
0.00	0.40	80.0	20.0
6.98	0.40	44.7	55.3
10.00	0.40	44.7	55.3
10.20	0.40	0.0	100.0
12.49	0.40	0.0	100.0
12.72	0.40	45.0	20.0
15.00	End	–	–

3. Results and discussion

3.1. Preliminary tests to exclude pore blocking and gel layer formation

Organic components, especially peptides in hydrolysates are likely to cause membrane fouling during ultrafiltration. Membrane fouling can be divided into three main types: adsorption, pore blocking and gel layer formation on the membrane surface. A reversibility test is one of the evaluation methods to exclude such fouling types. The reversibility test was done for the 1 kDa-Nadir membrane to evaluate fouling during filtration of 5 % (w/v) Prolastin (fish protein hydrolysate) at pH 5 (isoelectric point) [59,60].

For the filtration of the 5 % (w/v) Prolastin (fish protein hydrolysate), the steady state permeate fluxes increased when increasing transmembrane pressure (TMP) from 0 to 6 bar, as shown in Fig. 3 (blue arrow direction). After reversing TMP back from 6 to 4 and 2 bar, respectively (green arrow direction), the permeate flux was close to the flux at the same pressure beforehand. This excludes pore blocking and gel layer formation during the filtration. TMP and hydrolysate permeate flux were also constant during the filtration time of 45 min (Fig. S1., supplementary material, appendix S2). Moreover, the TMP values when filtrating water and the hydrolysate are similar (Table S6, supplementary material, appendix S2). These results support the absence of pore blocking and gel layer formation during the filtration of 5 % (w/v) Prolastin.

During the filtration of 5 % (w/v) Prolastin, the resistance values increased with increasing TMP (Fig. 4, gray bars). Increasing the pressure gradually builds up the (concentration) polarization layer in front of the membrane surface and reaches the maximum level at 6 bar. After reversing the pressure from 6 to 4 and then 2 bar, the resistance slightly dropped (Fig. 4, yellow bars). Even though the pressure was reversed to 4 and 2 bar, the resistance did not reduce because a cross-flow velocity of 0.1 m/s was too low to change the layer before the membrane surface. After flushing with demi-water, the resistance significantly reduced (Fig. 4, orange bar), but did not go back to the initial value of the cleaned membrane (Fig. 4, blue bar). This could be due to the very low cross-flow velocity and the strong stickiness of Prolastin. Finally, the membrane permeability was fully recovered after chemical cleaning (Fig. 4, green bar; Table S7; Fig. S2, supplementary material, appendix S2). This indicates no irreversible fouling.

The reversibility test was conducted to evaluate pore blocking and gel layer formation during filtrating 5 % (w/v) Prolastin at pH 5 using the 1 kDa-Nadir membrane, which has the smallest pore size (Table 1). The TMP and steady permeate flux values during the reverse pressure cycle (increasing TMP from 2 to 6 bar; then decreasing to 2 bar) exclude pore blocking and gel layer formation during the filtration time at all

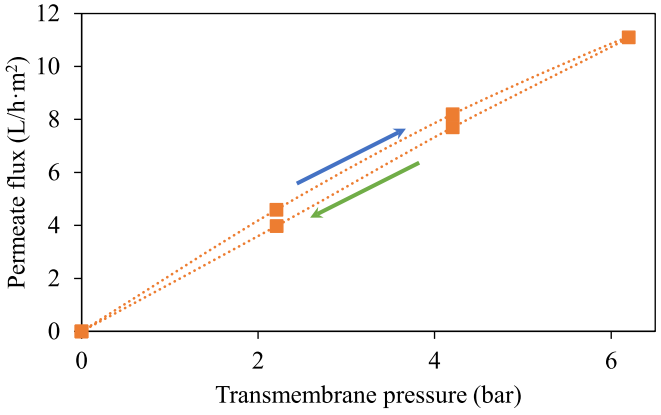

Fig. 3. Relationship between hydrolysate steady state permeate flux and transmembrane pressure for filtrating 5 % (w/v) Prolastin at pH 5 using the 1 kDa-Nadir membrane.

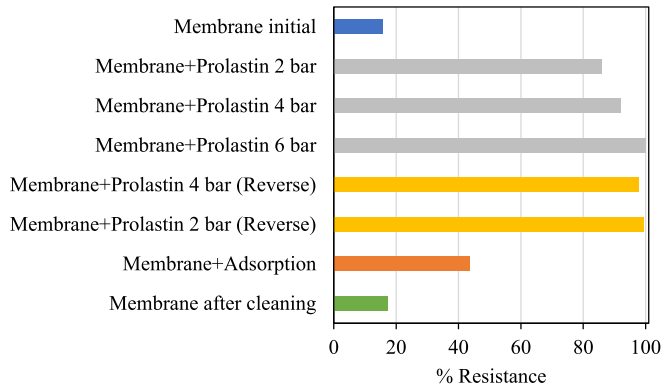

Fig. 4. Normalized resistance (%) before the filtration (membrane initial), during the filtration at various pressure (2, 4, 6, 4-reverse, 2-reverse), after water flushing (membrane + adsorption) and after chemical cleaning (membrane after cleaning).

pressures. Therefore, all filtration experiments in this study were operated under the assumption of no pore blocking and gel layer formation during the filtration time. The steady-state permeate flux and the TMP of a specific filtration condition were constant over the filtration time, which supports this assumption (see results in supplementary material, appendix S3, Fig. S3-S13).

3.2. Size-based UF separation of neutral and charged solutes

Four spiral wound PES membranes were used to fractionate the feed solutions containing neutral (dextran + MD) and charged (Prolastin) solutes with different concentrations. The uncharged case was done with a feed concentration of 0.5 % (w/v), whereas the solution concentration of the charged solutes was 5 % (w/v).

3.2.1. Volumetric permeate flux

Both membranes with a nominal MWCO of 5 kDa provided the same range of permeate fluxes, whereas the 1 kDa membranes exhibited rather different fluxes (Fig. 5). The permeate fluxes of the solutions with uncharged and charged solutes were lower than the water fluxes for all membranes, except the fluxes of the dextran + MD solutions treated by the 1 kDa-Nadir membrane (Fig. 5). A deviation of the solution flux from the water flux at higher transmembrane pressures implies the accumulation of solutes before the membrane surface, leading to concentration polarization. The fluxes of the 5 % (w/v) Prolastin solutions deviated significantly from the water fluxes compared to the dextran + MD fluxes. A solution of 5 % (w/v) Prolastin has a higher viscosity, which reduces the permeation rate. The charged solutes also have repulsive and attractive interactions between solutes and with the membrane surface.

Regarding the charge properties of solutes at the same feed concentration of 0.5 % (w/v), the permeate fluxes of the uncharged dextran + MD and the charged Prolastin solutions were comparable when using the 5 kDa-Synder membrane (Fig. 5, top left). This implies that the charge interactions within the polarization layer did not significantly affect permeation at a dilute concentration. However, increasing the concentration of Prolastin solution from 0.5 % to 5 % gave a significantly lower flux. This is most likely due to the increased solution viscosity. However, the effect of charge on the flux also depends on the type of membrane, as seen from the flux result of the 1 kDa-Nadir membrane (Fig. 5, bottom right). The fluxes of dextran + MD solutions were similar to the water fluxes at most pressures, but the Prolastin fluxes were much lower than those of dextran + MD solutions and water for all conditions.

3.2.2. Membrane retention

The comparison between the retention profiles of the neutral (Fig. 6, left) and charged (Fig. 6, right) solutes shows the effect of the solute properties on membrane retention. Overall, the retention profiles of the uncharged solutes were steeper and smoother (indicating a sharper separation cut-off) than those with the charged solutes. At the same feed concentration of 0.5 % (w/v), the 5 kDa-Synder membrane could slightly retain more molecules larger than 5 kDa of the charged solutes (R ~ 45 %) when compared to the neutral solutes (R ~ 40 %). Interestingly, an increase in Prolastin concentration from 0.5 % to 5 % clearly

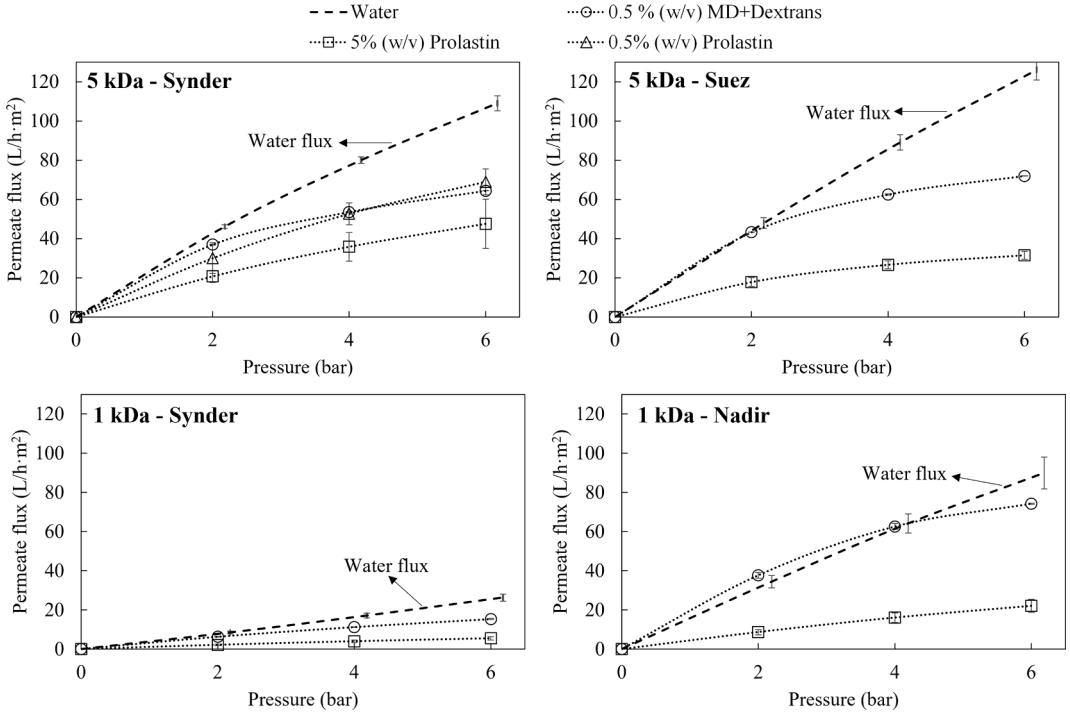

Fig. 5. Permeate water flux (—) and solution flux as a function of the pressure for 0.5 % (w/v) dextran + MD (○), 5 % (w/v) Prolastin (□) and 0.5 % (w/v) Prolastin* (Δ). The values are the averages of duplicate experiments. *The data was obtained from Chorhirankul et al. [43].

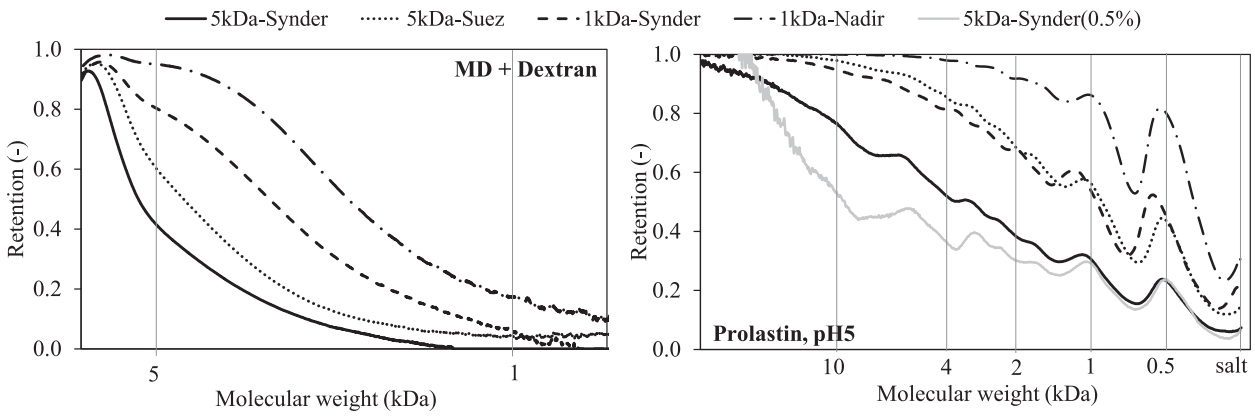

Fig. 6. Retention profile versus molecular weight of solutes from filtration at 2 bar. The feed concentrations of Dextran + MD and Prolastin (pH 5 = pI) were 0.5 % and 5 % (w/v), respectively. The grey line of the right figure is the retention profile of 0.5 % (w/v) Prolastin fractionated by the 5 kDa-Synder membrane (the data was obtained from Chorhirankul et al. [43]). All other lines represent 5 % Prolastin solutions.

enhanced the retention of the 5 kDa-Synder membrane. This suggests the impact of concentration polarization on membrane retention. Moreover, the retention of the 5 kDa-Suez appeared to be almost similar to the retention of the 1 kDa-Synder membrane when fractionating 5 % (w/v) Prolastin solutions. Therefore, membrane retention for charged solutes depends on not only solute sizes but likely also on other molecular properties that correspond to the molecular interactions within the concentration polarization layer at the membrane surface.

3.2.3. *Membrane permeability and membrane performance*

The retention factor was calculated from Equation (4) for each experimental condition. Then, all retention factors were averaged using Equation (7), as presented in Table 3. The average retention factor was plotted versus the average membrane permeability for all membranes, as shown in Fig. 7.

Membranes with the same nominal MWCO can be expected to have similar retention, especially with similar membrane materials. However, as shown in Fig. 7 and Table 3, the PES membranes with similar nominal MWCO specified by the membrane suppliers show significant differences in the average retention factors for both 1 and 5 kDa membranes (p value < 0.001). In addition, at similar nominal MWCO, the average retention factor of a membrane increased with a higher membrane permeability. Just based on pore sizes, one would expect the opposite. However, even though all membranes used in this study were PES membranes, we could not guarantee that all membranes had the exactly same materials for the membrane active layer and support layers. The significantly different permeabilities of the membranes with the same nominal MWCO (p value < 0.001, Table 3) indicate that the membrane characteristics are indeed not similar. The solute, feed concentration and operating conditions that membrane suppliers use to characterize membranes likely influence the specifications of the nominal MWCO by suppliers [61]. Therefore, this confirms that the nominal MWCO provided by membrane suppliers is not a good guideline for selecting membranes [43].

Table 3
Average water permeabilities of all membranes and average retention factors of the membranes when separating uncharged (Dextran + MD) and charged (Prolastin) solutes.

Membrane	Average water permeability, $L_{p,avg}$ (L/h·m^2·bar)	Average retention, R_{avg} (%) Uncharged solutes	Charged solutes
5 kDa-Synder	18.4 ± 0.5*	27.4 ± 0.8[a]	30.4 ± 0.3[a]
5 kDa-Suez	20.8 ± 0.9*	34.7 ± 1.1[a,b]	55.3 ± 2.6[c]
1 kDa-Synder	4.2 ± 0.3*	59.5 ± 0.3[c,d]	64.2 ± 1.7[d]
1 kDa-Nadir	14.8 ± 1.3*	78.5 ± 0.4[e]	80.3 ± 1.6[e]

[a, b, c, d, e] letters indicate the significant difference in R_{avg} values at p value $<$ 0.001 (One-Way ANOVA, Tukey's test).

Note: The results are the same set as shown in Fig. 7.

[*] $L_{p,avg}$ values are significantly different at p value < 0.001 (One-Way ANOVA, Tukey's test).

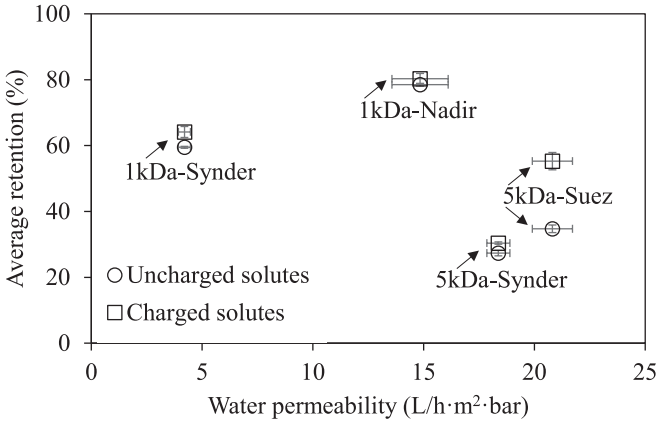

Fig. 7. Relationship between average water permeability and average retention of uncharged solutes, Dextran + MD (○) and charged solutes, Prolastin (□) for all membranes.

The effect of the electrostatic interactions from charged solutes on membrane retention was confirmed in our previous study [43]. The same 5 kDa-Synder membrane as used in this study was used to fractionate 0.5 % (w/v) Prolastin solutions at pH 5 and 8 with 0.6 M NaCl. The average retention factor for filtrating 0.5 % (w/v) Prolastin solutions was 27.4 ± 4.6 %, while the same membrane had an average retention factor of 27.4 ± 0.8 % when fractionating neutral solutes (dextran + MD) with a concentration of 0.5 % (w/v). Even though the average retention of both uncharged and charged solutes was thus similar, the difference in the standard deviations of both cases suggests that the membrane retention could be modified by adjusting the solution pH and adding salt. This means that the change in the charge states of solutes does affect the retention of the membrane at similar concentrations. The difference in the differential retention profiles between the neutral and charged solutes (Fig. 6) also supports these charge effects on membrane retention. Furthermore, the average retention factor of the 5 kDa-Synder membrane increased with increasing feed concentrations. While the value with 0.5 % Prolastin was 27.4 ± 4.6 %, it was 30.4 ± 0.3 % with 5 % (w/v) Prolastin. The increased retention factor with a

higher solution concentration might indicate stronger concentration polarization that hinders the permeation of solutes.

3.3. UF separation of charged solutes (peptides in a fish protein hydrolysate)

Electrostatic interactions significantly changed the separation when using UF membranes for fractionating charged solutes (*Section 3.2*). However, other molecular properties such as hydrophobicity and hydration, have not yet been examined. These properties may well lead to solute associating, changing their effective molecular weights, and may adsorb to the membrane, reducing pore size or blocking smaller pores, leading to smaller effective pore size. Consequently, we will assess the effect of these properties on the UF separation of a hydrolysate mixture.

Prolastin solutions with a concentration of 5 % (w/v) at various pH and ionic strength values were used. Although Prolastin contains peptides with different charges, the overall isoelectric point of the hydrolysate was assumed to be around pH 5. This is because the molecular weight distribution profile of Prolastin was comparable to the profile of a tuna black muscle hydrolysate [62] with an isoelectric point surrounding pH 5 [59,60]. This assumption was supported by the lowest buffering capacity of the retentate samples (comparable to feed) at pH between 5 and 6 where the charged molecules in the hydrolysate solutions contained the lowest amount of associating/dissociating groups (Fig. S19-S22, supplementary material, appendix S5). The pH conditions in this study were at pH 5 and 8 where the overall net charges of the hydrolysate solutions were neutral and negative. A polymeric membrane is typically negatively charged [63,64], and the zeta potential values of the membranes were reported as negative (see Table 1). The effect of charges was evaluated by adding 0.6 M NaCl to screen the electrostatic charges during separation.

3.3.1. Volumetric permeate flux

The water fluxes (dotted lines in Fig. 8) were similar to those shown in Fig. 5. The Prolastin fluxes obviously deviated from the water fluxes. A larger deviation of the solution fluxes from the water fluxes implies a thicker and more compact CP layer. In addition, the increase in the thickness and compaction of the CP layer decreased the charge effects on permeate flux, except for the 5 kDa-Synder membrane. Therefore, the characterization of the CP layer likely depends on the interaction between charged solutes and the membrane surface, which corresponds to the properties of membrane materials.

3.3.2. Membrane retention

Fractionation of 5 % (w/v) Prolastin solutions using the four different membranes gave different compositions of the permeates (Fig. 9). A smaller nominal MWCO membrane retained more peptides than one with a larger nominal MWCO when comparing the membranes from Synder. Thus, membrane retention is mainly based on size selection. However, the 5 kDa-Suez membrane had comparable retention as that of the 1 kDa-Synder membrane, as shown by the almost similar retention profiles (Fig. 6) and molecular weight distributions (Fig. 9).

Even though the chromatograms in Fig. 9 reflect fractionation based mainly on size exclusion, the peak profiles do not look identical for all membranes. Some shifted peaks imply a difference in the compositions of the permeate samples. This infers that some peptides might not permeate through the membrane. This is not only because of size, but also other selective mechanisms could be involved in the separation mechanisms. The buffering curves of the permeates (Fig. 10) support this conclusion. The difference in the buffering curves suggests the dissimilar compositions of the permeates obtained with the four membranes. A higher buffering capacity means the sample contained more associating/dissociating groups. The buffering curves (Fig. S19-S22, supplementary material, appendix S5) from other experimental conditions showed similar results.

Fig. 11 shows the influence of adjusting solution pH and ionic strength on retention profiles when using the four PES membranes for the fractionation of 5 % (w/v) Prolastin solutions. The retention profiles of the 5 kDa-Synder membrane and the 5 kDa-Suez membrane are clearly different. The profile of the 5 kDa-Synder membrane shows a rather gradual cut-off, while the 5 kDa Suez membrane is convex and shows a steeper cut-off at smaller molecular weights. To make this more concrete, the 5 kDa-Synder membrane showed 50 % retention of peptides larger than 4 kDa peptides, while the retention of the Suez membrane was above 80 % for these peptides. This indicates different interactions between charged peptides and the membrane surface. This is in agreement with Fig. 7 where a higher membrane permeability resulted in a higher retention of membrane. Both 1 kDa membranes also

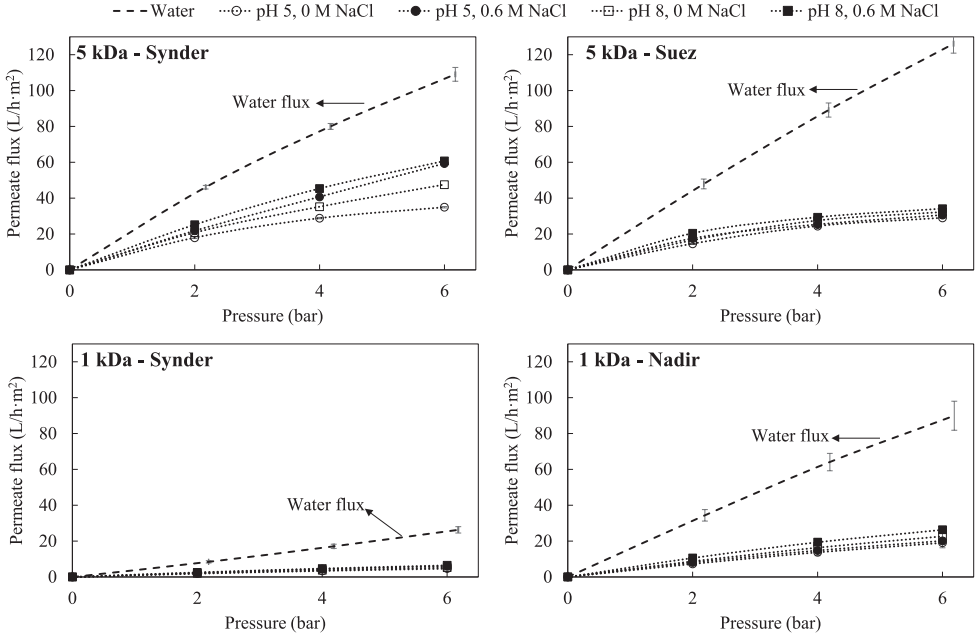

Fig. 8. Hydrolysate permeate flux as a function of the pressure at pH 5, 0 M NaCl (○); pH 5, 0.6 M NaCl (●); pH 8, 0 M NaCl (□) and pH 8, 0.6 M NaCl (■) for various membranes. The feed concentration of Prolastin hydrolysate was 5 % (w/v).

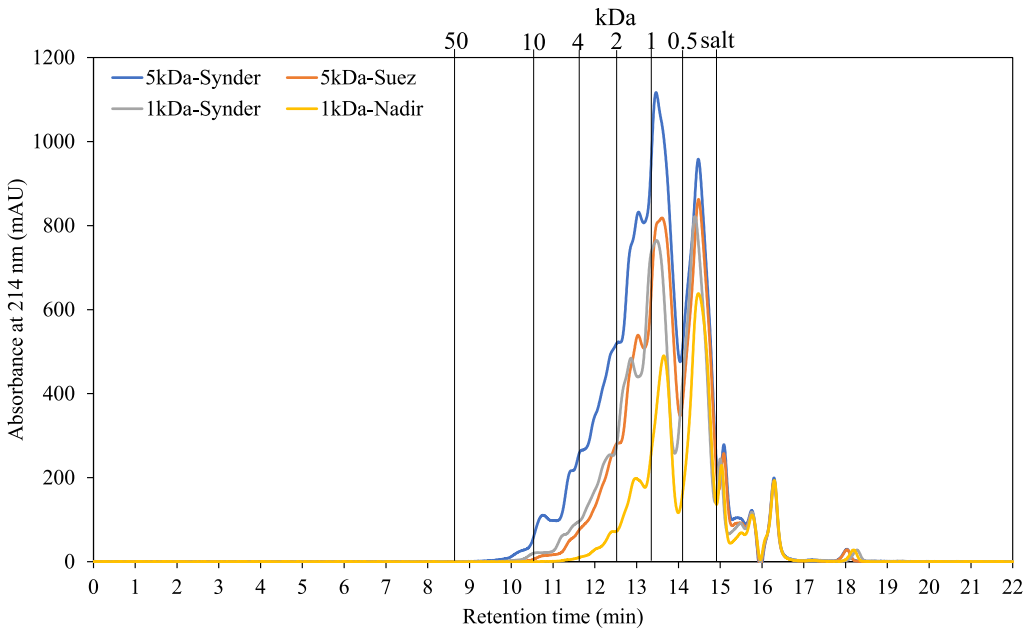

Fig. 9. Comparison of molecular weight distributions of permeate Prolastin samples fractionated at pH 5 and 2 bar.

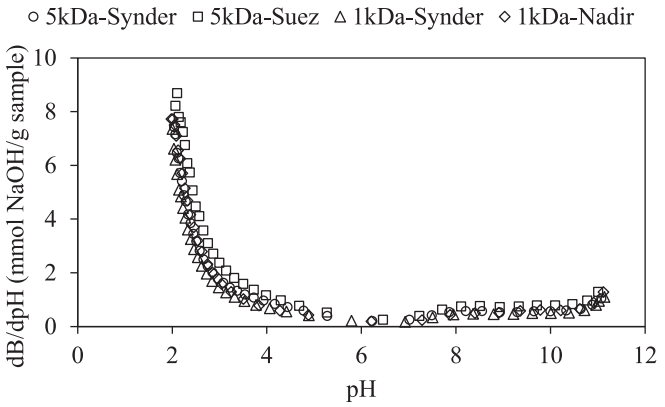

Fig. 10. Buffering curves of permeate Prolastin samples fractionated at pH 5 and 2 bar.

gave different retention profiles. Compared to a previous study with more dilute solutions [39], the charge effect in Fig. 11 is less pronounced. This is expected since higher concentrations also imply higher ionic strengths and, thus, better screening of the ionic interactions. Therefore, membrane retention mainly depends on membrane characteristics (implied by membrane permeability) when fractioning a mixture at a high concentration. The membrane characteristics likely relate to the formation of the concentration polarization layer that significantly determines membrane retention.

3.3.3. Amino acid profile of peptide fractions obtained from UF fractionation

An amino acid analysis was done to quantify the amount of the free amino acids and the amino acids obtained from hydrolyzing samples (free amino acids + peptides). The average free amino acid contents and the amino acid contents of hydrolyzed solutions for retentate samples were 6.3 ± 0.4 mM and 33.0 ± 1.5 mM, respectively. The amount of free amino acids in the retentate and permeate samples was mostly equal (Fig. S23, supplementary material, appendix S6), consequently assuming that all free amino acids passed through the membrane for all experiments. Therefore, the free amino acid contents were subtracted from the concentration of the amino acids in the hydrolyzed samples (free amino acids + peptides) to calculate the amino acid contents of peptides in the hydrolysate solutions.

Overall, the amino acid profiles of peptides in the retentate and permeate samples were similar, but the amino acid concentrations of peptides in the permeate were lower than those in the retentate. Fig. 12 (A) shows that all membranes gave quantitatively and qualitatively comparable amino acid profiles of peptides in the retentates. The amino acid concentrations of peptides in the permeates (Fig. 12, B) were reduced, evident in larger average retention factors of membranes (Fig. 7). In addition, the amino acid concentrations of peptides in the permeate are in accordance with HP-SEC profiles of Fig. 9. The result indicates that transport of specific peptides is not likely.

3.3.4. Retention of amino acid profile and hydration number

The retention factors of individual amino acids of hydrolyzed peptides from the retentate and permeate samples were calculated from Equation (3). These were related to the molecular weight, the amino acid residue molecular weight, the isoelectric point, the acid/basic dissociation constant, the hydrophobicity index and the hydration number. Most of them showed no relation. There is a very slight (negative) relationship only between the hydration number and the retention (Figs. 13-14). This implies that apart from size exclusion, the separation may also depend on peptide hydration. This supports adjusting the pH and adding salt into 5 % Prolastin solutions hardly changed the retention of membranes as shown in *Section 3.3.2*. The modification of the pH changed the overall net charge of the hydrolysate, but the functional groups of peptide structures stayed the same. Additionally, electrostatic screening by addition of salt may have slightly changed the effective hydrodynamic radius of the individual peptides and amino acids, affecting the retention.

The retention values of leucine, phenylalanine, methionine and histidine were outliers for most experiments (Figs. 13-14 and S29-S32, supplementary material, appendix S7). Fig. 12 shows that leucine was not detected in the permeates at all. The concentration of phenylalanine was also relatively low in the permeate samples. Thus, the 1 kDa membranes highly retain peptides containing leucine and phenylalanine as well as methionine. By contrast, the peptides consisting of histidine permeated freely through all membranes. The outliers could not be explained based on their molecular properties.

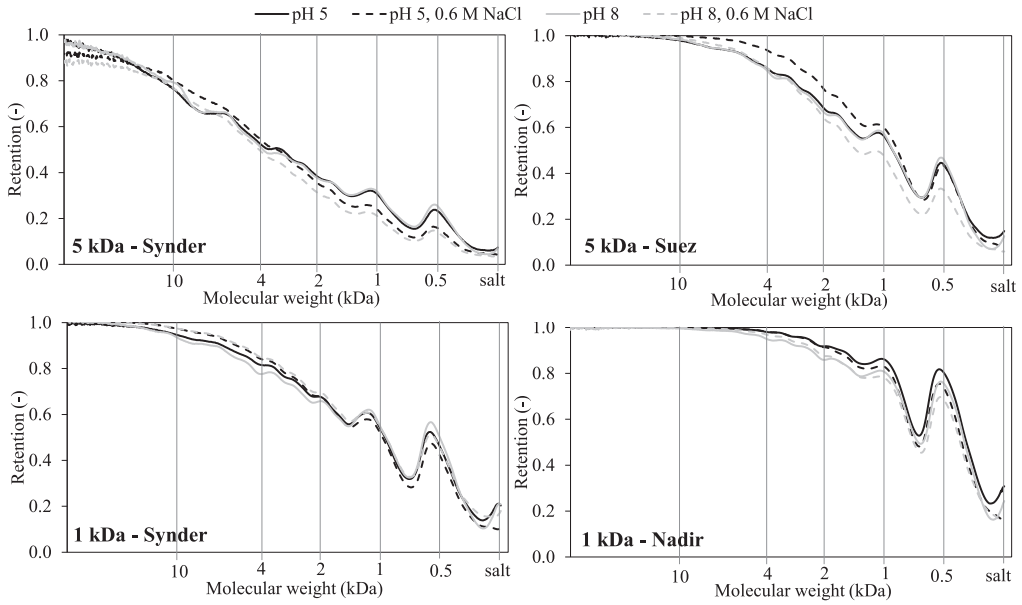

Fig. 11. Retention profile versus molecular weight of peptides during the Prolastin filtration runs through various membranes at 2 bar and different pH values and salt content: pH 5, 0 M NaCl; pH 5, 0.6 M NaCl; pH 8, 0 M NaCl and pH 8, 0.6 M NaCl. The overall retention and the retention of each peptide fraction can also be found in the supplementary material (Fig. S15-S18, appendix S4).

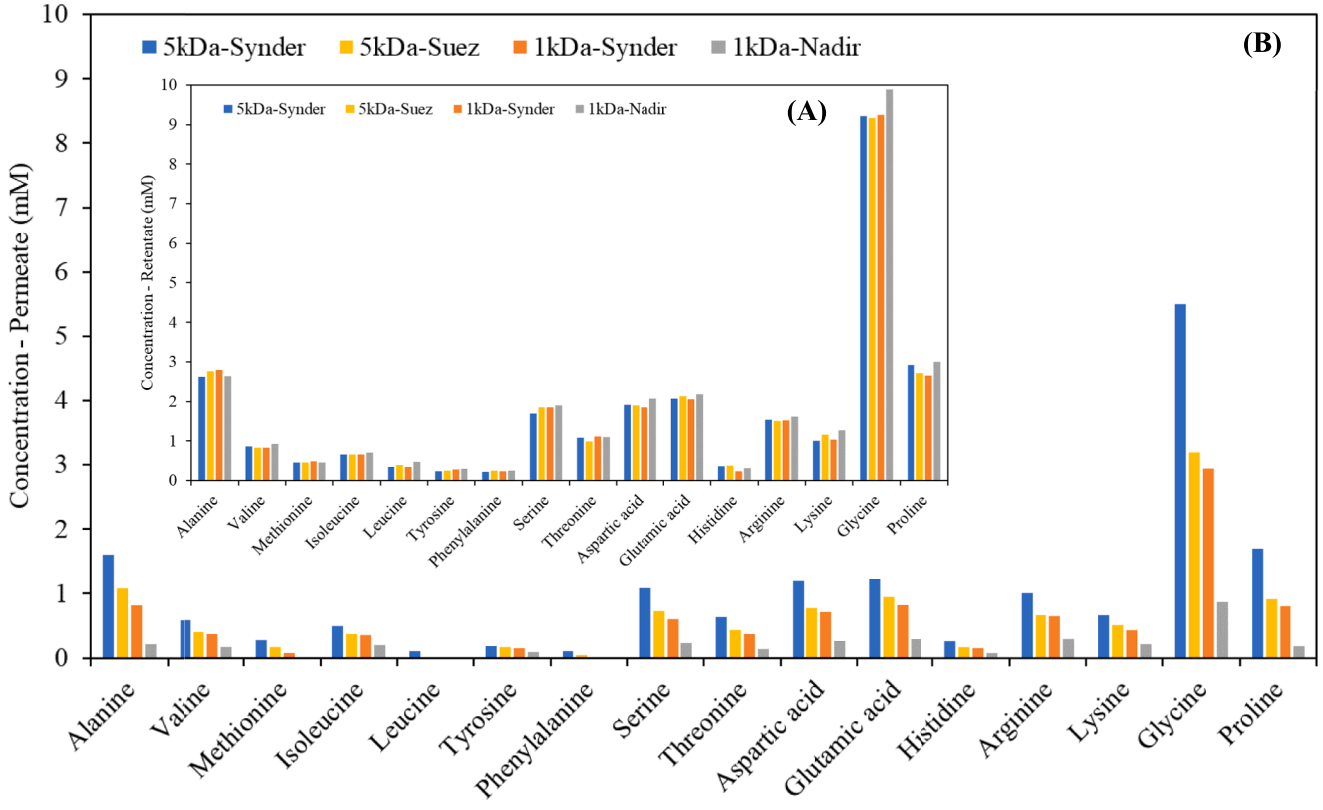

Fig. 12. Amino acid profiles of peptides of the retentate (A) and permeate (B) samples obtained from fractionating 5 % (w/v) Prolastin at pH 5 and 2 bar. *Note: Amino acid concentrations of peptides are the corrected concentrations by subtracting the concentrations of free amino acids from those amino acid concentrations of hydrolyzed samples (free amino acids + hydrolyzed peptides).*

The retention of amino acids did not relate to their side chain types (hydrophobic-aliphatic = pink; hydrophobic-aromatic = black; polar neutral = dark blue; acidic-charged = green; basic-charged = blue; unique = orange), as shown in Fig. 13. We found similar results for all membranes (Fig. 14 and S29-S32, supplementary material, appendix S7), indicating that this is a characteristic of the solution, not of the

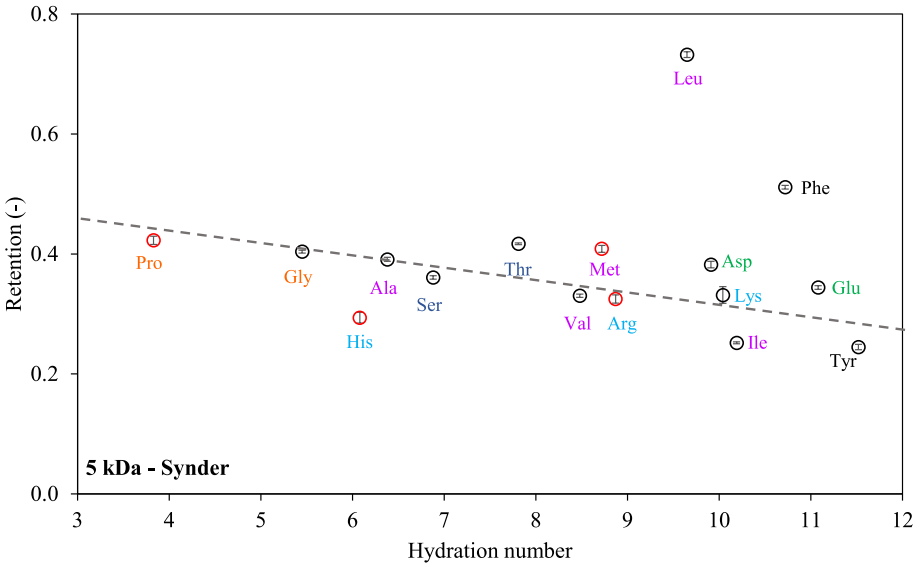

Fig. 13. Retention of amino acid profile of peptides as a function of hydration number. The peptides obtained from 5 % (w/v) Prolastin solutions that were fractionated by using the 5 kDa-Synder membrane at pH 5, 2 bar. Dotted line is for a guide for the eye. Pro = Proline; Gly = Glycine; His = Histidine; Ala = Alanine; Ser = Serine; Thr = Threonine; Val = Valine; Met = Methionine; Arg = Arginine; Leu = Leucine; Asp = Aspartic acid; Lys = Lysine; Ile = Isoleucine; Phe = Phenylalanine; Glu = Glutamic acid; Tyr = Tyrosine. *Note: Red circles (○) are the data with underestimated hydration numbers.*

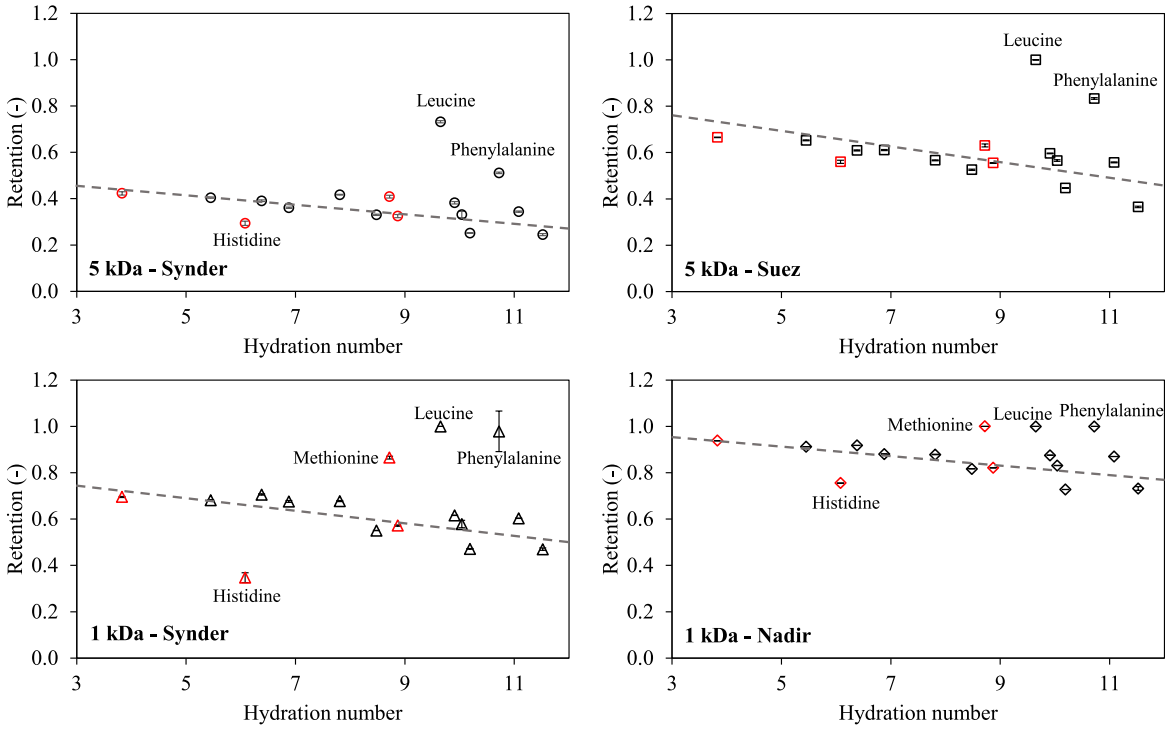

Fig. 14. Retention of amino acid profile of peptides as a function of hydration number. The peptides obtained from 5 % (w/v) Prolastin solutions that were fractionated by using various membranes at pH 5, 2 bar. Dotted lines are for a guide for the eye. Note: Red symbols are the data with underestimated hydration numbers.

membranes. The retention slightly decreased with increasing hydration number for all membranes. A larger hydration number reflects a higher number of water molecules binding to amino acid (peptide) molecules, which may screen any hydrophobic interaction between the solutes (which increases their effective molecular weight) and between the solutes and the membrane (pore) surface. Both effects could lead to easier permeation and lower retention.

4. Conclusions

Polymeric spiral wound UF membranes with the same nominal MWCO value do not always have identical membrane retention and selectivity for fractionating solutions with both neutral and charged solutes. In fractionating neutral solutes, membrane retention mainly depends on membrane characteristics that are not only membrane pore size but also porosity, tortuosity and membrane (active layer) thickness. With charged solutes, the interactions between solutes and the

membrane surface change the retention. A positive relation was found between permeability and retention of membranes with similar nominal molecular weight cut-off, with a higher permeability giving a higher retention of peptides. It is clear, however, that the nominal MWCO value specified by membrane companies, generally measured with neutral solutes, is not valid for solutions containing charged components. Prolastin (fish protein) hydrolysates show strong charge effects at lower concentrations but these are reduced at higher concentrations, as the charge effects are screened by the larger ionic strength. Consequently, the fractionation of the Prolastin hydrolysate was mainly based on size exclusion. While there was no clear relation between retention and most molecular properties of amino acids, a slight reduction of the retention was found with increasing hydration numbers for the amino acids present in the peptides. The peptides with leucine, phenylalanine and methionine were specifically retained by all four membranes, irrespective of the nominal MWCO, but allowed peptides with histidine to pass through easily.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2025.131726

Data availability

Data will be made available on request.

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