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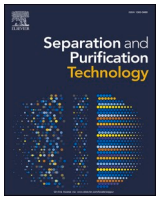

Electrophoretic removal of sinapic acid from rapeseed protein extract

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ABSTRACT

Isolation of proteins from oilseeds to supply functional proteins for the food industry is essential but challenging due to the presence of anti-nutrients such as phenolic compounds. To deliver proteins, the removal of phenolic compounds is crucial. Conventionally, this is accomplished by alcohol washing; however, this is resource-intensive, may be unacceptable for some and does not provide proteins with good techno-functional properties since it alters the native protein structure. To overcome such drawbacks, gentle processing methods must be developed. In this work, we investigated the electro-separation of sinapic acid from rapeseed protein extract. A porous medium (ion exchange or ultrafiltration membrane) permitting electromigration of only sinapic acid and retaining the proteins was utilized under two different potential differences. The electro-separation of sinapic acid relied on electrostatic and electrophoretic forces, which cause their adsorption and permeation. Among the treatments, 1.5 V over an anion exchange membrane showed the best performance, providing considerable sinapic acid removal (34.0 ± 4.0 wt%) while maintaining the protein content and pH stability. A larger system with a larger membrane surface area yielded as high as 90.3 ± 3.8 wt% of sinapic acid removal within 240 min while retaining 88.8 ± 7.6 wt% of the proteins.

1. Introduction

The emergence of plant materials as an alternative protein source is a result of the nutritional needs of the growing world population and the environmental impact of the livestock industry [1,2]. Besides, changes in dietary preferences due to health or moral issues also significantly impact this development [2]. On the other hand, transitioning to plant-origin proteins is not a trivial task, as it requires an understanding of the characteristics of different plant proteins and availability of suitable protein extraction methods, considering that plant matrices are complex in structure and comprise a range of non-protein materials [3].

Various plant sources from leaves to legumes have been proposed as a protein source [1]. Oilseeds are outstanding vegetative protein sources with an annual production volume of more than 650 MT (USDA, 2024). Industrially, their primary utilization is oil extraction; however, oilseeds also contain 20–30 wt% proteins, which are currently underutilized or even discarded. The main challenge in the extraction of proteins from oilseeds is the presence of phenolic compounds that interact with the proteins and form protein–phenolic complexes characterized by low solubility, reduced functionality, and indigestibility [4,5]. Besides, the presence of phenolic compounds affects the sensory properties by inducing a bitter–astringent taste, and a darker colour [1]. To obtain oilseed proteins of good quality and functionality, the removal of phenolic compounds, or dephenolization, is therefore crucial.

Phenolic compounds are generally eliminated using solvent extraction with high-polarity solvents like methanol and ethanol. Solvent extraction is a mature technology and is widely applied as it is simple and cost-effective [6]. However, it has some drawbacks regarding the obtained protein quality and efficiency. The use of alcoholic solvents alters the structure of the proteins and sometimes causes their denaturation [7,8,9]. Additionally, heat is often used to improve the extraction yield, and this can also degrade the proteins [10]. The conventional solvent extraction requires a large amount of solvents and recycling of the solvent, which requires long processing times and may release a residual amount of these greenhouse gases into the environment [11]. Finally, the use of ethanol may not be culturally acceptable to a sizable group of consumers. To mitigate the listed drawbacks and to deliver phenolic-free oilseed proteins, gentler and resource-efficient separation processes must be developed.

Ultrafiltration is an alternative to eliminate the organic solvent need

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in dephenolization by separating proteins and phenolics based on their size differences under applied pressure through a membrane. This process is followed by dialysis or diafiltration process for desalination of the concentrated protein solution [12,13,14]. An electric field can be utilized to enhance the separation, as it can be more effective than mechanical or thermal gradients and may contribute to (i) improved selectivity by acting only on charged compounds and (ii) simultaneous desalting as a result of electromigration of ions [15,16,17]. The use of electric field has already been investigated for the removal of tobacco polyphenols using electrodialysis [18]. Nevertheless, to the best of the authors' knowledge, there is no study yet on electrophoretic dephenolization of oilseed protein extracts.

In this work, separation using an electric field over an ion exchange membrane and/or ultrafiltration membrane was used to remove sinapic acid (SA), the most abundant free phenolic acid in rapeseeds, from rapeseed proteins [19]. Under the influence of an external electric field, charged compounds, SA and the proteins in this case, electromigrate towards the oppositely charged electrode. Both SA and the proteins carry a negative charge under alkaline conditions; however, the rapeseed proteins are much larger molecules (12–300 kDa) than SA (0.224 kDa) [20,21]. Thus, separation can be obtained using a membrane that only allows the electromigration of SA and retains the proteins. The retention and permeation of SA and its removal were evaluated under various conditions. Additionally, the protein loss and pH stability was investigated.

2. Materials and methods

2.1. Materials

Alizze variant (*Brassica napus*) rapeseeds were kindly provided by a seed breeder to be used for protein extraction. Sinapic acid (purity $\geq$ 99.0) and all chemicals used were of analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA). Deionized water (Milli-Q, Merck Millipore, Darmstadt, Germany) was used to prepare all solutions and dispersions.

2.2. Extraction of rapeseed proteins

Rapeseed proteins were extracted from dehulled and defatted rapeseed meal by dispersing 100 g of rapeseed meal in deionized water at a ratio of 1:10 (w/w). The pH of the dispersion was then adjusted to 9.0 by adding 1.0 M NaOH. The dispersion was stirred at 400 rpm for 4 h at room temperature. Subsequently, the dispersion was centrifuged at 10,000 g and 4 °C for 30 min (Sorvall Lynx 4000 Centrifuge, Thermo Scientific, USA). The supernatant containing the extracted proteins was collected and freeze-dried (Epsilon 2-10D LSCplus, Martin Christ, Germany). To remove the co-extracted phenolic compounds, the dried protein extract was washed four times for 1 h with excess methanol under constant agitation. At the end of each cycle, the mixture of methanol and protein extract was centrifuged at 10,000 g and 4 °C for 30 min, and the pellet was collected and redispersed in methanol until the four washing cycles were completed. Finally, the dephenolized rapeseed protein extract was dried overnight under a fume hood and stored at −20 °C for further analysis.

2.3. Protein content of the extracted proteins

The total protein content of the obtained rapeseed protein extract was measured using the Dumas method (Rapid N exceed, Elementar, Germany). For the analysis, aspartic acid was used as a standard and oxygen (O_2) served as a blank sample. To quantify the total protein content, nitrogen conversion factor of 5.7 was applied. The analysis was conducted in triplicate, and the results were expressed as mean ± the standard deviation (wt%).

2.4. Total phenolic content of the extracted proteins

The total phenolic content (TPC) was determined using the Folin Ciocalteu assay. For the analysis, 1.0 mL of sample was mixed with 5.0 mL of deionized water, and 0.5 mL of Folin Ciocalteu reagent was added. The mixture was vortexed, and 1.0 mL of 20 wt% Na_2CO_3 solution was added. Finally, the volume of the mixture was completed to 10.0 mL by adding deionized water. The samples were incubated in dark for 1 h, and their absorbance was measured at 725 nm using an UV–Vis Spectrophometer (DR6000, Hach, Colorado, U.S.A). Tannic acid was used as a standard for the quantification. All analyses were done in triplicate and the results were given as the mean ± standard deviation (wt%).

2.5. Sinapic acid content of the extracted proteins

To determine the SA content, high-performance liquid chromatography (Ultimate 3000 RS UHPLC system, Thermo Scientific, MA, U.S.A.) equipped with a UV–Vis detector was used. As a stationary phase, Gemini® 3 μm C18 110 Å column (150 × 4.6 mm, Phenomenex, CA, U.S.A) was used. The mobile phase consisted of eluent A (0.1 % trifluoroacetic acid in deionized water) and eluent B (0.1 % trifluoroacetic in acetonitrile), introduced to the column at a flow rate of 1.0 mL/min. For the analysis, a gradient elution program was applied. The program started with 95.0 % A and 5.0 % B and gradually transitioned, first to 85.0 % A and 15.0 % B over 4 min, followed by a change to 80.0 % A and 20.0 % B within the next 10 min, then to 50.0 % A and 50.0 % B over the following 10 min, and finally, returned to the initial composition in the last 3 min of analysis. The column temperature was maintained at 35 °C, the UV detector was set to 220 and 325 nm and the injection volume was 10 μL. The system was calibrated using sinapic acid (purity $\geq$ 99.0) with different concentrations between 0.01 and 0.2 mg/mL for the quantification. All analyses were done in triplicate, and the results were given mean ± standard deviation (wt%).

2.6. Electrophoretic removal of sinapic acid from the rapeseed protein extract

2.6.1. Electrodialysis cell configuration

Electro-separation of SA from the rapeseed proteins was performed using an electrodialysis (ED) system. The system includes an ED cell (length 20 cm, width 10 cm, 84 cm^2 of effective surface area), three peristaltic pumps to circulate the solutions (flow rate: 2.0 L/h), buffer solution (1.0 mM potassium phosphate buffer at pH 8.0, flow rate: 2.0 L/h) and electrolyte solution (0.5 M Na_2SO_4, flow rate: 20.0 L/h), and a power supply (PLH250 DC Power Supply, AimTTi, Huntingdon, U.K.) connected to the electrodes on both sides of the ED cell. The ED cell contains stacked ion exchange membranes (IEM) (Shandong Tianwei Membrane Technology Co., Ltd., Shandong, China) separated with specific flow spacers to form separate compartments for the electrodes, the sample (retentate) and the buffer (permeate). Both electrodes (anode and cathode) were separated from the sample and the buffer solution using cation exchange membranes (CEM), and the retentate and the permeate cells were separated using either an anion exchange membrane (AEM) or an ultrafiltration membrane (UFM) (VT, Synder, CA, U.S.A). Further details regarding the specifications of the used membranes are provided in Table 1.

2.6.2. Sample preparation

To prepare the sample solution, dephenolized rapeseed protein extract and SA were mixed at a ratio of 20:1 w/w and dispersed in 1.0 mM potassium phosphate buffer (pH 8.0). Since the addition of SA decreased the pH, this was adjusted to 8.0 prior to the separation experiment using 0.1 M NaOH and 0.1 M HCl solutions.

2.6.3. Measurement of the limiting current density

The limiting current density (LCD) is an important parameter that

Table 1
Specifications of ion exchange and ultrafiltration membranes used in the experiments.

Property	Anion Exchange Membrane (AEM)	Cation Exchange Membrane (CEM)	Ultrafiltration Membrane (UFM)
Ion exchange capacity (mmol/g)	0.90–1.10	0.90–1.10	–
Thickness (wet) (μm)	40–50	40–50	–
Water Uptake (25 °C) (wt%)	15–20	15–20	–
Electrical resistance (Ωcm^2)	≤2.50	≤3.00	–
pH stability	1–12	1–12	1.8–11
Temperature stability (°C)	15–40	15–40	Up to 55
Material	Polystyrene	Polystyrene	Polyethersulfone
Molecular weight cut-off (kDa)	–	–	3
Surface charge sign	Positive	Negative	Negative

determines the highest current value to maintain an Ohmic linear relation between the current and potential difference for electrodialysis processes. To measure the LCD, the method by Isaacson and Sonin was used [22,23]. To do so, the potential difference was incrementally increased from 1.0 to 3.0 V, and the corresponding current value was recorded for each potential difference. Then, the current density (A/m^2) and the potential difference (V) values were plotted to determine the LCD value, marking the point where the relation between voltage and current density changes.

2.6.4. Operation of the electrophoretic separation

Electrophoretic removal of SA from the rapeseed protein extract was performed using an electrodialysis (ED) device equipped with ion exchange and/or ultrafiltration membranes. For the separation, both electrophoresis and size differences between SA and the rapeseed proteins were utilized. The mixture of SA and proteins was introduced to the ED cell near the cathode (negatively charged) electrode, causing their electrophoresis direction towards the anode (positively charged) electrode. Due to their size differences, only SA passed through the membrane and was thus removed (Fig. 1).

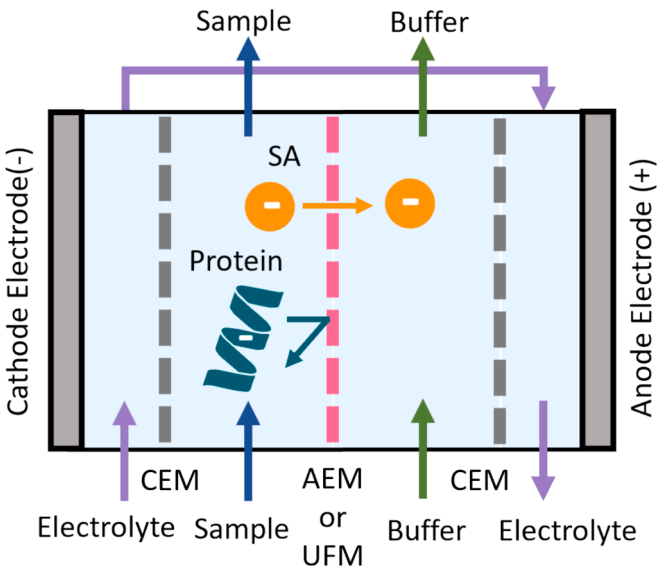

Fig. 1. The schematic design of the electrodialysis device for electrophoretic sinapic acid – rapeseed proteins separation. Purple, blue, and green arrows indicate the path of the electrolyte, sample and buffer solution in the electrodialysis cell. CEM: Cation exchange membrane, AEM: Anion exchange membrane, UFM: Ultrafiltration membrane, SA: Sinapic acid. Buffer is 1.0 mM potassium phosphate buffer at pH 8.0 and the electrolyte solution is 0.5 M Na_2SO_4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.6.5. Evaluation of the electrophoretic separation of sinapic acid from rapeseed protein extract

The electromigration of SA through the membranes was quantified by measuring both the retained and permeated SA content. For this, the HPLC method specified in Section 2.5 was used. The SA content, flux and removal (wt%) were calculated using Equations (1), (2) and (3), respectively.

$$Sinapic\ acid\ content(mg) = Sinapic\ acid\ concentration(mg/mL) \bullet Sample\ volume(mL) \quad (1)$$

$$Sinapic\ acid\ flux(mg/m^2h) = \frac{Permeated\ sinapic\ acid\ content(mg)}{Surface\ area\ of\ membrane\ (m^2) \bullet Duration(h)} \quad (2)$$

$$Sinapic\ acid\ removal\ (wt\%) = \frac{Initial\ sin.acid\ content\ (mg) - final\ sin.acid\ content\ (mg)}{Initial\ sinapic\ acid\ content\ (mg)} \bullet 100 \quad (3)$$

The separation experiments were performed both below and above the LCD using AEM and UFM as separation membranes. The experiments lasted for 240 min, and samples from the retentate and permeate cells were collected at certain time points (0, 30, 60, 120, 180 and 240 min). Besides, pH and conductivity were measured using a multimeter (HQ440d multi, Hach, Tiel, Netherlands) at the specified time points to monitor the changes, while the volume and temperature of the retentate and permeate were measured at the beginning and end of the experiments.

The protein concentration in the collected samples was also quantified to assess the loss during the process. For this purpose, again High-Performance Size Exclusion Chromatography (HPSEC) (Ultimate 3000 UHPLC system, Thermo Scientific, MA, U.S.A.) equipped with a UV detector was used. A combination of a TSKgel G3000S (WxL 7.8 mm × 300 mm) and a TSKgel G2000S (WxL 7.8 mm × 300 mm) (Tosoh Bioscience LLC, King of Prussia, PA, U.S.A.) served as a stationary phase, and 30 % acetonitrile in deionized water and 0.1 % trifluoroacetic at a constant flow rate of 1.5 mL/min was used as a mobile phase. The column temperature was kept at 30 °C, the UV detector was set at 214 nm and the injection volume was 10 μL. The system was calibrated using purified rapeseed protein in the range of 0.03–2.0 mg/mL. The total

protein content (mg) was calculated using Equation (4):

$$Total\ protein\ content(mg) = Protein\ concentration(mg/mL) \bullet Sample\ Volume(mL) \quad (4)$$

After evaluating the separation, the most optimal condition, which yielded the highest SA removal, the lowest protein loss and stable pH, was studied for complete SA removal from the mixture.

2.7. Statistical analysis

Statistical analysis was carried out by using SPSS version 25.0 for Windows (IBM Corp. NY, USA) to assess the significance of differences in SA and protein content throughout the processing and among different treatments. For this purpose, one-way ANOVA and Tukey test were used. The results were evaluated at a 95 % confidence level.

3. Results and discussion

3.1. Characterization of the rapeseed protein extract

Proteins were extracted from defatted rapeseed meal and dephenolized to be used as a protein fraction in the separation experiments. For their characterization, the total protein content, total phenolic content and SA content were determined. The dephenolized rapeseed proteins contained 69.76 ± 0.41 wt% of proteins. To evaluate the efficiency of methanol washing, TPC and SA content were determined before and after the dephenolization step. The initial TPC of 5.35 ± 0.13 wt% decreased to 2.43 ± 0.10 wt% after fourfold washing with methanol. SA, on the other hand, was almost completely removed (0.01 ± 0.00 wt%) from the protein extract, indicating that the treatment was effective in obtaining a SA-free protein extract. It should be noted that the Folin Ciocalteu method may not be entirely accurate in measuring the total phenolic content, as some amino acids like tyrosine can also react with the Folin reagent and interfere with the results [24,25].

The mixture of SA and proteins was prepared based on TPC in the unwashed protein extract to achieve a similar composition for the separation experiments. Therefore, the original ratio of TPC to the protein extract of 1:18.7 w/w was rounded up 1:20 w/w to prepare the mixture of SA and dephenolized protein extract. Here, SA represented phenolic compounds originally found in the rapeseed protein extract.

3.2. Electrophoretic sinapic acid removal from the rapeseed protein extract

To assess the electro-separation under different conditions, both AEM and UFM were tested below and above the LCD, which was 0.40 ± 0.10 A/m^2 with the corresponding potential difference of 1.8 ± 0.2 V for AEM and 0.03 ± 0.00 A/m^2 with the corresponding potential difference of 2.0 ± 0.0 V for UFM, respectively (Fig. S.1-2, Supplementary Information). Therefore, 1.5 V was chosen to represent the separation below the LCD for both membranes. 1.0 V per membrane (3.0 V in total) was used for the measurement above the LCD, which was also the maximum allowed potential difference for the ED cell. Using the listed four different combinations (1.5 V AEM and UFM, and 3.0 V AEM and UFM), SA retention and permeation were analyzed. Fig. 2 summarizes the

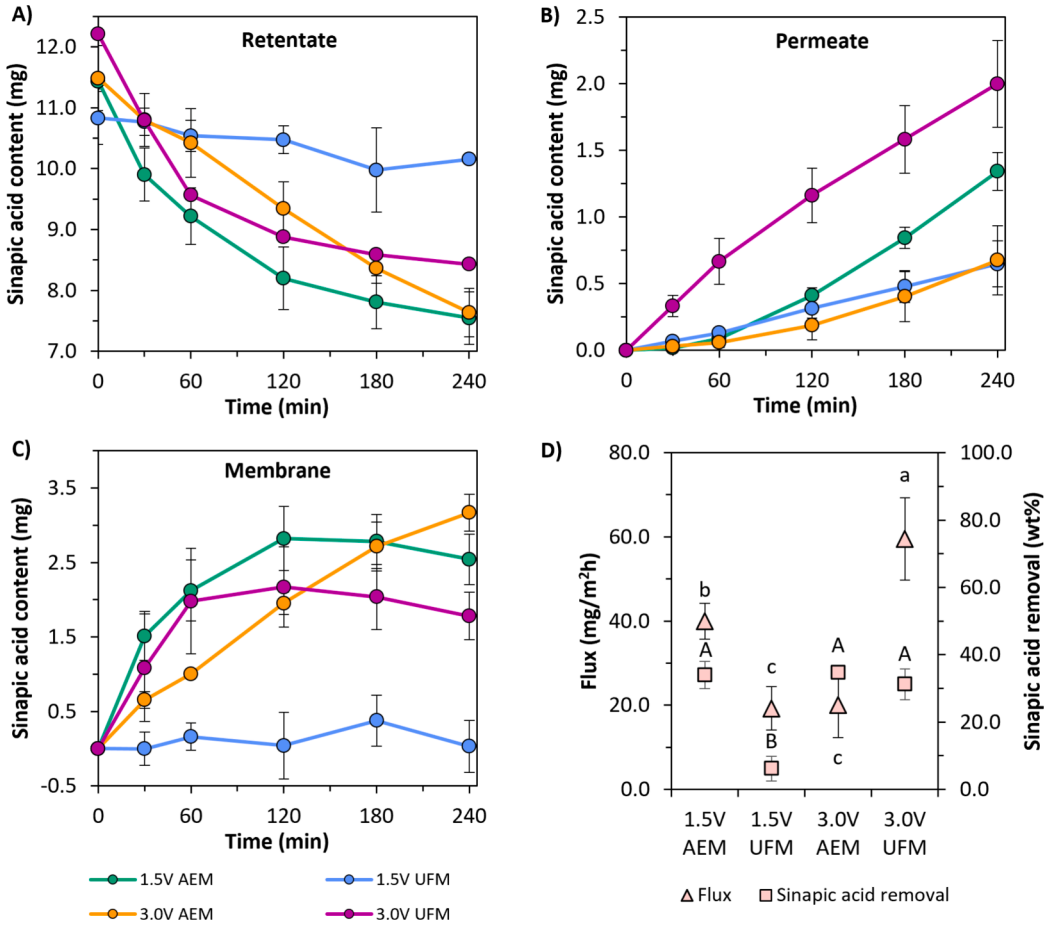

Fig. 2. Sinapic acid content in the retentate (A), permeate (B) and in the membrane (C), and flux and sinapic acid removal (wt%) (D) under the treatments of 1.5 V AEM, 1.5 V UFM, 3.0 V AEM and 3.0 V UFM. AEM: Anion exchange membrane, UFM: Ultrafiltration membrane. Small letters indicate significance between the flux values and the capital letters indicate significance between the sinapic acid removal under different treatments ($P < 0.05$).

changes in SA content in the retentate (A), permeate (B) and membrane (C), respectively. Furthermore, Fig. 2D shows the permeation flux and total removal of SA from the mixture.

In the case of using 1.5 V potential difference (0.11 ± 0.02 A/m^2 average current density) with an AEM, the highest SA reduction was observed within the first 120 min, after which the reduction rate decreased. This may stem from the adsorption rate of SA in the AEM. Fig. 2C indicates that the maximum adsorption of SA also took place at 120th min, after which the adsorption of SA began to decrease, due to the continuing extraction towards the positive electrode. The charged compound (SA) was initially adsorbed by the membrane as a result of electrostatic attraction due to the opposite electrical charge of SA (−) and the membrane surface (+), along with the electrophoretic forces. Then, the adsorbed SA permeated into the buffer. Adsorption occurred faster than permeation as the electrophoresis rate of a charged compound is faster in an aqueous environment than in a porous membrane [26,27]. This demonstrates that the adsorption in the AEM does not impede the continuing removal of SA. Even though the electrophoretic and electrostatic forces were the most significant forces in the SA migration, co-current electroosmotic flow, which occurs as a result of the migration of attracted counter-ions by the membrane toward the anode electrode, and diffusion could also contribute to the SA migration.

The observed mechanism below the LCD using 1.5 V and an AEM, however, changed when the LCD was exceeded. At 3.0 V (0.68 ± 0.10 A/m^2 average current density), adsorption due to the enhanced electrophoretic force played a more significant role than electromigration in SA removal. This can be attributed to the decrease in transport number of SA. The transport number (T_i) defines the ratio of the current carried by ion i to the overall current (I_t) carried by all ions through the membrane. Its value depends on the fraction of the ion i (α_i) and the total current ($T_i = \alpha_i \bullet I_t$). Given that, the transport number decreases when the fraction of the ion i decreases under the constant current. When surpassing the LCD, the salt flux across the membrane increases, accelerating desalination. A more rapid desalination was indeed observed during the experiments (Table S.1, Supplementary Information). This leads to a decrease of the fraction of the other compounds migrating through the membrane, such as SA. Hence, the permeability of SA decreased. Besides, formation of H^+ and OH^- ions from water splitting reactions might have also contributed to the decrease in the transport number of SA [28,29,30,31]. In addition to the potential decrease in SA transport number, its enhanced electrostatic interactions with the charged groups in the membrane due to the increasing pH could influence its adsorption. Due to the complexity of an ED system operating under over-limiting current conditions, revealing the exact underlying causes is challenging. In general, operating the system below the LCD is advised.

When an UFM instead of an AEM was used, a different permeation mechanism was observed. With 1.5 V (0.02 ± 0.00 A/m^2 average current density), no significant change in SA content was obtained. Only a small fraction of SA passed through the membrane, and there was almost no discernable sorption. This highlights the importance of the electrostatic interaction between the compounds to be transferred and the membrane. The surface charge of the UFM is negative, giving rejection of negatively charged compounds like SA when other forces, diffusive and electrophoretic forces, are not sufficient. Besides, the use of a negatively charged membrane caused a counter-current electroosmotic flow, which may also have impaired the electromigration of SA. As a result of these effects, SA was rejected by the membrane even though its size (0.24 kDa) is much smaller than that of the molecular weight cut-off of the membrane (3 kDa). Similar to the SA migration, no significant desalination in the retentate was observed (Table S.1). Here, one should note that the average current density in the case of 1.5 V UFM was much lower due to its higher resistance compared to the case of 1.5 V AEM, which could also cause low SA permeation.

SA permeation through the UFM was, however, visible with an increased potential difference of 3.0 V (2.65 ± 0.90 A/m^2 average current density). The electrophoretic force now overcame the electrostatic repulsion and electroosmosis. Consequently, SA could be adsorbed by the membrane and enter the permeate. Doyen et al. [32] reported an increasing permeation of charged compounds through a UFM when exposed to a higher electric field due to their enhanced electrophoresis rate. It is assumed that the different reactions to the overpotential state in AEM and UFM are due to their permeability and selectivity. The generated OH^- ions and other anionic compounds were transferred through the AEM as long as their size was small enough; however, not all OH^- ions produced in the retentate cell were carried to the permeate cell when using the UFM, which resulted in an increasing pH and conductivity in both compartments (Table S.1 and S.2). This observation may indicate that the repulsion forces between OH^- or anionic salt ions and the UFM were stronger than those between SA and UFM. Therefore, the majority of the current may still be carried by SA, resulting in a higher permeability.

In general, the results demonstrate that a significant reduction in SA content at the end of 240 min ($P < 0.05$) under all treatments except application of 1.5 V UFM. While all three treatments yielded comparable SA removal ($P > 0.05$), they exhibited different SA permeation profiles and fluxes. In ED, SA removal was achieved by adsorption and permeation. The highest permeation flux (59.5 ± 9.7 mg/m^{2}h) was observed at 3.0 V using a UFM as a result of the increased electrophoretic forces dominating the electrostatic repulsion and electroosmosis. The second highest SA flux (39.9 ± 4.2 mg/m^{2}h) was observed with an AEM using 1.5 V. In this case, the flux was enhanced by the electrostatic attraction forces between SA and the membrane, as well as by the electroosmotic flow.

The lowest flux values were observed with 3.0 V using an AEM (20.1 ± 7.8 mg/m^{2}h) and with 1.5 V using a UFM (19.3 ± 5.1 mg/m^{2}h). When the AEM was used, exceeding the LCD enhanced salt and OH^- ions transport through the membrane led to a reduction in the transport number of SA and its permeation. For the UFM, the electrostatic repulsion forces and reverse electroosmotic flow prevented adsorption of SA and its permeation due to inadequate electrophoretic forces generated by 1.5 V potential difference.

In addition to SA removal, the retention of the protein in the feed is important. The protein content was tracked throughout the process for each applications, and the results were represented in Fig. 3. Only the

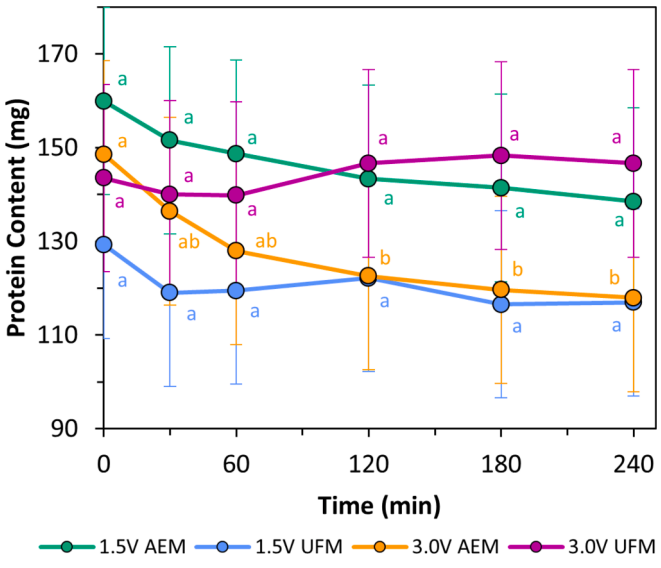

Fig. 3. Changes in the protein content under different treatments throughout the process. Time = 0 min indicates the initial protein content. AEM: Anion exchange membrane, UFM: Ultrafiltration membrane. The letters indicate significant difference in the protein content during 240 min for each treatment ($P < 0.05$).

application of 3.0 V with an AEM caused a significant protein loss ($P < 0.05$). The initial protein content of 148.6 ± 16.1 mg decreased to 117.9 ± 4.6 mg at the end of 240 min. This is consistent with the increasing SA adsorption by the membrane due to the increasing electrophoretic forces. Like SA, rapeseed proteins were also negatively charged under alkaline conditions. Therefore, they also migrate toward the anode electrode and adsorb to the positively charged membrane surface. This effect was also observed with 1.5 V using an AEM; nevertheless, here the protein loss was not significant within 240 min ($P > 0.05$). In UFM applications, there was almost no protein loss as a result of the electrostatic repulsion between the proteins and the membrane surface ($P > 0.05$). The protein content data also reveals that the initial protein content (time = 0 min) varied, and there were considerable level of standard deviation even within the same treatment, like 1.5 V AEM. This variation occurred because the dried protein extract after methanol wash contained some stiff flocculates, preventing the collection of a uniformly dispersed protein sample for each analysis.

Lastly, all treatments were assessed on their pH stability. As a general rule, water splitting reaction takes place when the current exceeds the LCD, leading to pH changes as a result of generated H^+ and OH^- ions [33]. The results confirmed this. The initial pH of 8.0 was stable throughout the process when the UFM was used at 1.5 V, while a small deviation was observed when the AEM was used; the final pH was 7.77 ± 0.11 in the retentate and 7.64 ± 0.14 in the permeate cells. The reason of this is the transport of anions through the AEM, which was not visible with the UFM due to the electrostatic repulsion.

On the other hand, strong pH changes were observed with both AEM and UFM as soon as the LCD was exceeded. In AEM, the H^+ and OH^- ions produced in the retentate cell migrated through the CEM and AEM (Fig. 1), and the pH decreased to 6.14 ± 0.45 in the retentate cell. However, the passage of OH^- ions through the AEM caused the pH to increase to 9.78 ± 0.64 in the permeate cell. As mentioned earlier, ion transport through the UFM was not that efficient; therefore, OH^- ions accumulated in both retentate and permeate cells, which caused an increasing pH in both compartments. The final pH was recorded as 10.49 ± 0.30 in the retentate and 10.62 ± 0.63 in the permeate cells. The detailed pH information can be found in the supplementary information (Table S.2).

3.3. Upscaling the electrophoretic sinapic acid removal process

Overall, four different configurations were tested to reveal the most optimal treatment for performing the electrophoretic SA separation from rapeseed protein extract. Regarding the total SA removal, protein loss and pH stability; the treatment of 1.5 V AEM is the most promising condition. To demonstrate an enhanced SA removal under this condition, the approach was upscaled using five AEMs.

Changes in the mixture composition when treated by five AEMs and 1.5 V can be seen in Fig. 4A. The majority (84.5 ± 2.7 wt%) of SA was removed within the first 120 min, and the total removal was 90.3 ± 3.8 wt% at the end of 240 min. Besides, no significant protein loss was observed during the processing and 88.8 ± 7.6 wt% of proteins was retained in the sample solution ($P > 0.05$). Nonetheless, when using five AEMs, the SA flux decreased to 12.9 ± 3.8 mg/m^{2}h from 39.9 ± 4.2 mg/m^{2}h when a single AEM was used. This reduction in flux is attributed to the reduced potential difference per membrane. The overall potential difference was kept constant at 1.5 V to show the effect of the increased surface area, resulting in less electrophoretic forces and impaired permeation. However, the increasing SA content in the permeate indicated that the separation obtained was not based solely on adsorption but both (transient) adsorption and permeation of SA (Fig. 4B).

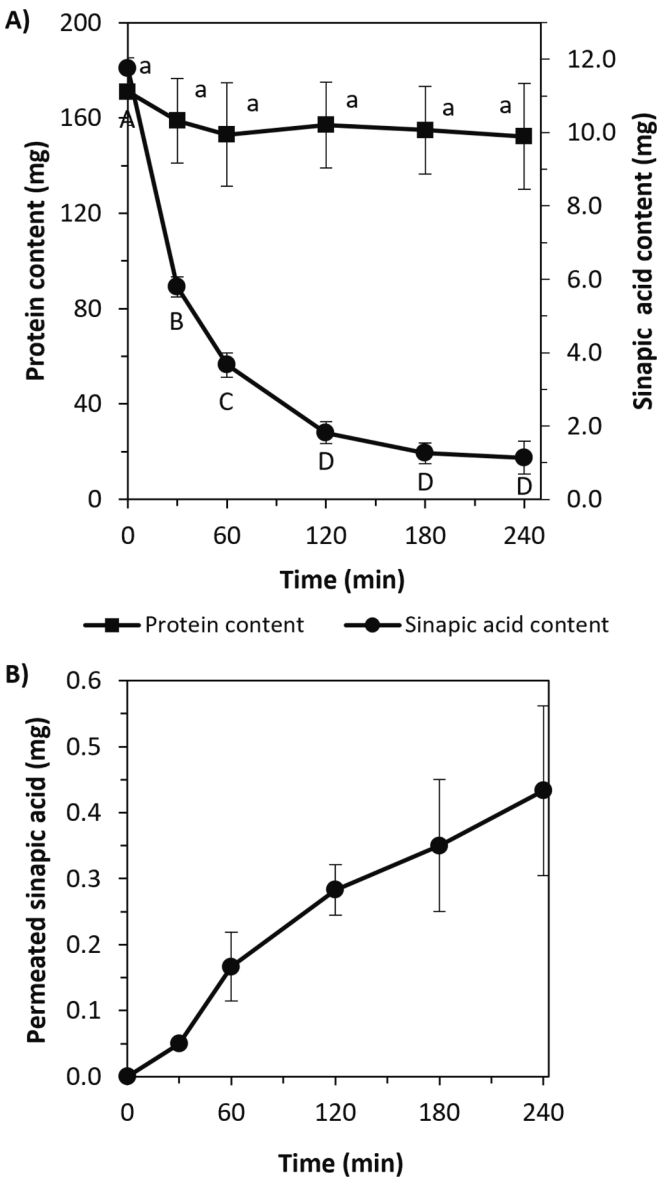

Fig. 4. A) Changes in the protein (■) and sinapic acid content (●) of the sample when treated by 1.5 V and five anion exchange membranes. Time = 0 indicates the initial protein and sinapic acid content. Small letters indicate significance in the protein content and the capital letters indicate the significance in sinapic acid content during the processing B) Permeated sinapic acid content.

4. Conclusions

The electrophoretic removal of SA relied on transient adsorption in the membrane and continuous permeation into the permeate chamber, driven by electrophoretic and electrostatic forces. The electrostatic forces enhanced the separation when positively charged AEM was used, but impaired the SA electromigration in the UFM due to its negatively charged surface under the influence of 1.5 V. Consequently, 34.0 wt% and 6.2 wt% SA removal were recorded in AEM and UFM, respectively. This situation changed under the over potential situation. Exceeding the LCD enhanced the desalination rate, reducing the SA transport. Nevertheless, a similar level of SA removal (33.5 wt%) with 1.5 V was obtained, highlighting the effect of enhanced electrophoretic forces. Under 3.0 V UFM treatment, both SA migration and adsorption increased due to the suppressed electrostatic repulsion forces, and 31.0 wt% SA was removed.

Only the application of 3.0 V AEM induced a notable protein loss due the combination of the electrophoretic and electrostatic attraction forces. Additionally, the pH was maintained stable only when the system was run under the LCD, both 3.0 V AEM and UFM applications resulted

in dramatic pH changes due to water splitting reactions and transport of OH^- ions.

To demonstrate the improved SA separation, an AEM was chosen with 1.5 V as it provided a significant decrease in SA content, no significant protein loss and a relatively stable pH. To upscale this approach, five AEMs were stacked to form five flow channels and increase the membrane surface area. With this configuration, 90.3 wt% SA was removed in 240 min.

Overall, the principle of electrophoretic SA removal was demonstrated utilizing an electrodialysis device. The results indicate that electric field-driven separation of SA from the proteins as a result of electrostatic and electrophoretic forces is possible and can be scaled up by increasing the membrane surface area.

CRediT authorship contribution statement

Kübra Ayan: Conceptualization, Investigation, Writing – original draft. **Remko M. Boom:** Conceptualization, Supervision, Writing – review & editing. **Constantinos V. Nikiforidis:** Conceptualization, Supervision, Writing – review & editing.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2024.130215.

Data availability

Data will be made available on request.

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