Recovery of biomolecules from marinated herring (Clupea harengus) brine using ultrafiltration through ceramic membranes

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A B S T R A C T

Marinated herring processing brines, which are usually discarded, are rich in salt, protein, non-protein nitrogen, iron, fatty acids, antioxidant and even possess enzymatic activity. This study investigated the performance of ceramic ultrafiltration of two herring spice brines with a major focus on recovery of high value biomolecules such as proteins, fatty acids, minerals, and phenolic compounds. Chemical and biological oxygen demand (COD, BOD5) as well as total suspended solids (TSS) were also measured to follow the performance of the ultrafiltration. The retentates contained 75–82% (<62.7 mg/mL) of the protein and 75–100% of the fatty acids compared to the level in the initial brines. The nitrogen concentration was approximately halved in the permeate, whereas the phosphorous content was significantly increased in the permeate compared to the initial brines. Moreover, a retention of up to 42% COD, >95% TSS and >85% iron was obtained using the ceramic membranes. The two permeates generated were both fat-free and contained approx. 2% of the proteins compared to the unfiltered brines, and the retention of the phenolic compounds were ranged from 0 to 39%. The results presented in this work demonstrate that ceramic ultrafiltration can recover biomolecules from marinated herring brines although pre-filtration optimization is still needed.

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1. Introduction

In the production of marinated herring, a traditional Scandinavian product, the volumes of wastewater generated can reach more than 700 L per 100 kg herring produced; of which up to 100 L is generated as brine during the final maturation step. This wastewater is usually pooled and treated at the “end of the pipe” without any attempt to collect the organic matter or recycle the water. The costs for disposal of waste and wastewater and the environmental regulations are two major driving forces for introducing separation and treatment technologies. However, the recovery of (high) valuable biomolecules for human, animal or industrial uses is, in many cases, the secondary purpose (Waldron, 2007), but have recently received increasing focus. In a review by Olsen, Toppe, and Karunasagar (2014) the use of by-products as new resources is discussed and it is stated that today only a few high-value products are on the market. They point at an overestimation of the market, small volumes of high quality by-products, and high cost related to purifying biomolecules of interest. Nonetheless, before they even reach the industrial waste streams, marinated herring brines are food grade and follow hygienic standards. Therefore, it might be realistic to extract valuable compounds from these liquid effluents.

In the treatment of wastewater, a promising technique is membrane filtration; either ultrafiltration (UF) alone or in combination with e.g. microfiltration (MF) (Afonso, Ferrer, & Börquez, 2004). Using UF, one can separate high molecular weight (HMW) components, like proteins and suspended solids, from low molecular weight (LMW) components, like mono- and disaccharides, amino acids, inorganic acids, or sugars and salts (Wagner, 2001). Afonso et al. (2004) studied an integrated process of MF and UF for the concentration of proteins from fish meal effluents, which resulted in a recovery of 69% of the proteins. Such recoveries may

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allow for additional revenue and a significant reduction of environmental pollution. Cassini, Tessaro, Marczak, and Pertile (2010) demonstrated that the use of ceramic UF in the pre-treatment of isolated soy protein wastewater has great potential, as they managed to retain 34% chemical oxygen demand (COD), 52% protein, and 86% total suspended solids (TSS).

Ceramic membranes made from silicon carbide (SiC) have recently been certified for use in the food industry, and according to the manufacturer these membranes are extremely hydrophilic, thus very efficient in oil/water separation. Besides this, they are chemically inert and tolerate high salt content and temperature. With these properties, ceramic membranes might offer a better separation of the herring brines, compared to traditional synthetic polymer membranes. One serious problem in UF is membrane clogging and pre-treatment is therefore usually required. Electro-flocculation (EF) is an electrochemical pre-treatment method, which has been shown to be a low-cost technique in e.g. harvesting marine microalgae (Lee, Lewis, & Ashman, 2013) and in separating solid and dissolved pollutants from wastewater (Mollah, Schennach, Parga, & Cocke, 2001). EF utilizes electric current through the use of a ‘sacrificial’ anode that releases iron or aluminium ions through electrolytic oxidation. These coagulant ions lead to aggregation of suspended particulate matter, and as the process also produces hydrogen bubbles the result is flocculation (Ben-Sasson & Adin, 2010). Some of the reported advantages of EF are clear, colourless and odourless water, easy separation of flocs, and no need for chemicals (Mollah et al., 2001).

In an attempt to separate and collect high valuable biomolecules from marinated herring brines into HMW and LMW fractions, we have here combined EF with UF to evaluate if this could provide a feasible and unique separation setup that has not been previously tested within seafood processing. In a recent study we analysed brines from four different marinated herring products, and showed that they are rich in dry matter, salt, protein, non-protein nitrogen, iron, fatty acids, antioxidant activity as well as peroxidase and protease activity (Gringer, Osman, Nielsen, Undeland, & Baron, 2014). Two brines contained spices, and although the composition of the spice mixture is confidential, it is likely that the documented antioxidant activity was a result of spice-derived polyphenols (Shahidi & Zhong, 2010). For this reason, the brines tested in this work were from spice-cured (SC) and traditional barrel-salted spice-cured (TSp) herring products.

The objective of the present work was thus to evaluate the performance of either EF or a synthetic polymer membrane i.e. a polypropylene filter (PF) pre-treatment in combination with ceramic UF for the fractionation of TSp and SC into HMW retentate and LMW permeate and to investigate the partitioning of the high value biomolecules into these fractions; e.g. proteins, fatty acids, and phenolic compounds whilst water quality indicators were also monitored (COD, BOD$_5$ (biological oxygen demand in 5 days at 20 °C) and TSS).

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade, purchased from Merck (Darmstadt, Germany) or Sigma–Aldrich (Steinheim, Germany). All water used was double deionized water.

2.2. Treatment of samples

2.2.1. Herring brine

Brines from the last step in the production of marinated herring (*Clupea harengus*) were supplied from Lykkeberg A/S (Hørve, Denmark), and prepared according to Lykkeberg A/S production protocols. Two brines were obtained; from spice-cured fillets (SC), and from traditional barrel-salted spice-cured herrings (TSp). For SC, fillets were ripened for ~190 days. TSp results from salting whole herring for 200 days whereafter filleting takes place and the fillets are placed back in the brine for additional ripening (~1 year). The brines have been previously characterised (Gringer et al., 2014) and contain 14–16% salt, 41–57 mg/mL protein and 4–9 mg/mL lipids. Furthermore, they also contain a considerable amount of sugar and spices, however the levels are confidential. Brines were treated on-site using the tested technologies and generated samples were stored at ~80 °C until further analysis.

2.2.2. Electro-flocculation (EF) and polypropylene filter (PF)

For EF, a pilot scale unit (Fig. 1, point (3)) was provided by Application Factory A/S (Hørsholm, Denmark), consisting of a polypropylene housing with inlet at one bottom corner and outlet at the opposite top. Inside, a number of aluminium plates were placed, through which the process water was flowing. Flow rate was 1000 mL/min and 180A was applied. Foam/floc were produced which was manually collected. The outlet of EF (EF-OUTLET) was drained 5 cm from the bottom (to avoid the sediment particles) into the collecting tank (4), and used as inlet for the UF unit.

For membrane filtration, a dead-end 50 µm polypropylene filter (PF) (6) was used (Heco Filtration A/S, Hedensted, Denmark). Filtration was carried out until complete clogging of the filter, ~2 h. Outlet of PF unit was retentate (PF-RET) and permeate (PF-PER), of which the latter was inlet to UF.

2.2.3. Ultrafiltration (UF)

UF tubular ceramic membranes made of silicon carbide (SiC) were supplied by LiqTech International A/S (Ballrup, Denmark). The membrane was Ø 25 mm × 305 mm with 31 × 3 mm round channels. Filtration area was 0.09 m$^2$, and nominal pore size 0.040 µm. This system, shown in Fig. 1, consisted of a collecting tank (100 L) (4), a recirculating pump (7), and the UF membrane inside a stainless steel housing (9). The feed solution (EF-OUTLET or PF-PER) flowed along the length of the membrane and was divided into permeate (13), or retentate which returned to the collecting tank (4). The system operated in cross flow mode (2 m/s) with three pressure transmitters (0–6 bar), placed at the module entrance (8), at the concentrate outlet (10) and at the permeate outlet (12). The transmembrane pressure was controlled by the retentate valve (11) and the recirculating pump (7), and was kept at 2–2.6 bar. Process time was kept at exactly 1 h, after which both permeate (UF-RET) and retentate (UF-PER) was collected. During UF the temperature increased from 5/7 °C to 24/26 °C for the different brines. Efficiency of the membrane is described by the retention (%), $R$, given by the difference in the concentration (C) of the compound of interest in permeate and in inlet water:

$$R = \left(1 - \frac{C_{\text{permeate}}}{C_{\text{inlet}}}\right) \cdot 100\%$$

2.3. Methods

2.3.1. Proximate composition

pH was measured using a Metrohm 827 pH-meter (Herisau, Switzerland). Salt content (wt%) was measured according to AOAC standard method (2005). Dry matter (DM) content was measured by a two-step evaporation of water (60 °C for 24 h plus 105 °C for 24 h), and calculated as (mass of dry sample*100)/(mass of wet sample). Ash content (%) was measured by burning at 600 °C for
24 h, and reported as \(\frac{\text{mass of ash} \times 100}{\text{mass of wet sample}}\).

Protein content (mg/mL) was obtained using the BCA kit (Thermo Scientific, Pierce, Rockford, USA), with bovine serum albumin (BSA) as standard. Fatty acid analysis was completed by extraction with chloroform and methanol (Lee, Trevino, & Chaiyawat, 1996) followed by methylation and detection by gas chromatography mass spectrometry, GC-MS (Cavonius, Carlsson, & Undeland, 2014). Chromatographic parameters were previously described by Gringer et al. (2014). Total fatty acid (mg/mL) was calculated as the sum of all detected fatty acids, using C:17 as standard.

2.3.2. Trace elements

In the analysis of iron, zinc, calcium and magnesium, 0.75 mL of concentrated nitric acid, 0.15 mL of concentrated hydrochloric acid and 5 mL sample were mixed in Teflon vials. Samples were digested (Ethos plus milestone microwave, Sorisole, Italy) with a temperature increase from 20 to 180 °C in 15 min and subsequently kept at 180 °C for 20 min. Samples were cooled to room temperature, decanted into test tubes and diluted to 10 mL using water. Analysis of iron and zinc was conducted by ion chromatography according to Fredrikson, Carlsson, Almgren, and Sandberg (2002). Analysis of calcium and magnesium was performed using Atomic Absorption Spectroscopy (Agilent Technologies, Kista, Sweden). Quantification was conducted using iron and zinc standards (Fluka, Buchs, Switzerland) and calcium and magnesium standards (Ultra Scientific, Rhode Island, USA), and reported in µg/g. Nitrogen content (mg/g) was determined by Kjeldahl method, according to AOAC standard method (1995). Total phosphorous content (mg/L) was measured by photometric cuvette tests from Hach Lange (LCK349, Bronshøj, Denmark) and measured on a high-performance VIS spectrophotometer at 800 nm (DR3900, Hach Lange, Denmark). Aluminium content was measured by complete digestion and subsequent analysis by inductively coupled plasma mass spectrometry, ICP-MS (Perkin–Elmer SCIEX, ELAN 6000). For sample digestion, 2 g of brine was mixed with 5 mL HNO₃ (69%), 3 mL H₂O₂ (30%) and 0.5 mL HCl (37%), and digested (10 min) in a microwave (Multiwave 3000, Anton Paar, Austria) at 1400 W, according to Anton Paar Application Notes. Prior to ICP-MS analysis water was added to a final volume of 10 mL. Aluminium content (mg/L) was calculated from a standard curve with CentiPUR Aluminium standard solution (Merck, Darmstadt, Germany).

2.3.3. Water quality indicators

COD and BOD₅ were determined by photometric cuvette tests from Hach Lange (LCK014 and LCK555, respectively), measured on the DR3900 spectrophotometer mentioned above at 605 and 620 nm, respectively and reported in g/L. TSS were determined according to Danish Standard (1985), and reported in g/L.

2.3.4. Phenolic compounds and oxidation

Total phenolic compounds (TPC) were determined according to Farvin and Jacobsen (2013). In short, 100 µL brine was mixed with 0.75 mL 10% Folin-Ciocalteu reagents and incubated at room temperature for 10 min. 0.75 mL 6% sodium bicarbonate was added and incubated at room temperature for 90 min in the dark. Subsequently, the samples were vortexed and centrifuged (3 min at 13,684 g) and the absorbance read (Synergy 2 Multi-Mode Microplate Reader, BioTek® Instruments, Inc., Vermont, USA) at 725 nm on 200 µL. A calibration curve was made with gallic acid (GA) and TPC is reported as mg gallic acid equivalents (GAE)/mL brine:

\[
\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}} = \frac{A_{0\text{lg GA/mL}} \times \text{slope of calibration curve}}{\text{dilution factor}}
\]

Oxidation was measured by determining the concentrations of thiobarbituric acid reactive substance (TBARS) according to Sørensen and Jørgensen (1996) and adjusted for sugar (Du & Bramlage, 1992). This method is preferred as it gives a good result.
general indication of the oxidative status and it is known to correlate well with sensory evaluation. In short, 5 mL of supernatant (3 min at 13,684 g) were homogenized with 30 mL of 7.5% trichloroacetic acid (TCA) containing 0.1% propyl gallate (PG) and 0.1% ethylenediaminetetraacetic acid, disodium salt (EDTA) for 30 s in an Ultra Turrax blender (9500 rpm, IKA® T25, Staufen, Germany) and filtered through a Whatman filter no. 42. Five mL of the filtrate was mixed with 5 mL 0.02 M thiobarbituric acid (TBA) solution and incubated at 100 °C for 40 min, before reading the absorbance at: 440, 532 and 600 nm, using ε at 532 nm of 1.57 × 10^5 (Albro, Corbett, & Schroeder, 1986) for malondialdehyde (MDA), and the molar absorbance (MA) of sucrose at 440 and 532 nm of 147 and 8, respectively (Du & Bramlage, 1992). Non-specific turbidity was extracted at 600 nm. TBARS values are given as MDA equivalents (MDAeq) in nmol MDAeq/mL brine:

\[
\frac{(A_{532} - A_{600}) - (A_{440} - A_{600}) \cdot \left( \frac{MA_{\text{of sucrose}}}{MA_{\text{of sucrose}}} \right)}{157000} \cdot 10^6
\]

2.4. Statistical analysis

Measurements were carried out in triplicates unless otherwise stated. Results are given as means ± standard deviations. The GraphPad Prism® software Ver. 4.03 was used with P < 0.05 and results were compared using one-way ANOVA with Tukey's post-test.

3. Results and discussion

3.1. Pre-treatment

Electrofiltration (EF) with aluminium ions or membrane filtration with a dead-end 50 μm polypropylene filter (PF), were evaluated as methods of pre-treatment of TSp and SC prior to ceramic UF in order to analyse the partitioning of the high value biomolecules in HMW retentate and LMW permeate.

For EF-tests with TSp, 73 kg TSp RAW BRINE was used of which 20.3 kg was dry matter, 10.4 kg was ash, 5.1 kg was protein and 0.7 kg was fatty acids. The retention of molecules provided by EF is given in Table 1 and show, that dry matter, ash and protein were retained to the same degree; 47, 46 and 49%, respectively. The fatty acids were, however, retained to 79%. Running SC through the same pre-treatment, the amount of SC RAW BRINE used was 119.4 kg of which 28.8 kg was dry matter, 17.3 kg was ash, 6.3 kg was protein and 0.3 kg was fatty acids. In comparison with EF-treatment of TSp, subjecting SC to EF gave rise to slightly higher retentions regarding dry matter, ash and protein, 55–58%, whereas the fatty acid content was retained to the same extent. Testing with PF, the starting amounts were 37.6 kg and 37.0 kg RAW BRINES TSp and SC, respectively, and the compositions the same as in the EF-trials. In both cases, the retention of dry matter, ash and protein were between 34 and 37%. The retention of fatty acids was 51% and 79% for TSp and SC, respectively. The amounts of EF-OUTLET and PF-PER, were approximately 49% and 67%, of starting RAW BRINES, respectively, for both TSp and SC, and thus EF resulted in less inlet for the subsequent UF treatment compared to PF. On the other hand, it should be noted that EF was more time efficient than PF. Others have shown that EF is a time efficient and low cost technique, for example Ben-Sasson and Adin (2010), who reported a significantly decrease (up to 90%) in filtration-energy consumption in dead-end microfiltration using aluminium-based EF as pre-treatment. Xu, Sheldon, Larick, and Carawan (2002) tested aluminium-based EF on the wastewater from egg processing for recovery and utilization of this by-product. They recovered high concentrations of protein (36–50%) and fat (32–42%) in the generated sludge/floc, which is comparable with the values found in our work, although the fatty acid retention was even higher for our brines (78–79%). Anfson et al. (2004) studied the protein recovery from fish meal effluents by microfiltration followed by ultrafiltration. During the microfiltration step (decreasing pore size: 80, 20 and 5 μm) they obtained a retention of 19% protein and 94% fat. These results were thus 1.8 times higher (34–35%) in terms of protein retention and 1.1–1.8 times lower (51–79%) in terms of fat retention compared to our study, which may be due to different composition of effluents used in their study.

As described in Section 2.2.2, EF makes use of a ‘sacrificial’ anode that releases aluminium ions of which some end up as part of the flocs, whilst others will sediment at the bottom of the equipment or even solubilize in the EF-OUTLET. Exposure of aluminium has been described in connection with development of e.g. Alzheimer’s disease (Bondy, 2014) hence is considered a health hazard. Additionally, in 2007 the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2007) developed a provisional tolerable weekly intake for aluminium of 1 mg/kg body weight. Thus, as the main purpose of our work was to evaluate the recovery of high-value biomolecules from TSp and SC after pre-treatment with e.g. EF, it was necessary to investigate the level of aluminium ions in the EF-OUTLET. The aluminium content reported in Table 2 clearly shows that not all aluminium ended up in the floc or sediment during EF, but was solubilized in the EF-OUTLET and was present in both UF-PER and UF-RET in significantly (P < 0.05) higher concentrations than after PF. The highest concentrations were detected in UF-RETs after EF treatment of TSp and SC, with values of 1371 and 1688 mg/L, respectively. The amount of aluminium present in the permeates represent approximately 12–14% of the amount present in the retentates and was 5 and 25 times higher when compared to the PF-PERs for TSp and SC. On the basis of these results, EF using an aluminium electrode is not appropriate as a pre-treatment for recovery of high-value biomolecules from marinated herring spice brines, thus only PF was further considered.

3.2. Ceramic ultrafiltration

As a second step after PF, UF was conducted with ceramic membranes. A mass balance was carried out to evaluate the

<table>
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<th>Table 1</th>
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<tr>
<td>TSp</td>
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<td>EF-OUTLET</td>
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<tr>
<td>Dry matter</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Protein</td>
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<td>Fat</td>
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Values are calculated on mean (n = 3) values from inlet and permeate samples.
consecutive treatment of PF and UF (Table 3). It shows that the UF-PERs (generated from 25.2 and 24.9 kg TSP and SC PF-PERs, respectively) only amounted to 1.2 and 1.6 kg, respectively, i.e. 4.8% and 6.4%, which is probably due to membrane clogging which also impacted the flux (~20 L/m²/h). Still the fatty acids were retained by 100% by the ceramic membrane, which was due to the hydrophilicity of the membrane. The retention of dry matter and ash was 95.5% and 94.3% for TSP and 94.7% and 94.3% for SC, respectively. Proteins were retained by 98.8% and 98.2% for TSP and SC, respectively. The reported dead volume in the pilot plant was approximately 5 L, but with a continuous process under industrial conditions no dead end volume would be expected.

Afonso et al. (2004) studied the protein recovery from fish meal effluents by consecutive microfiltration and ultrafiltration. The UF alone resulted in 62% retention of the proteins (15 kDa molecular weight cut-off (MWCO)), and the integrated process of microfiltration and UF enabled 69% retention of the proteins. Dumay, Radier, Barnathan, Berge, and Jaquen (2008) used a 10 kDa MWCO cellulose membrane for the recovery of proteins and lipids from sardine surimi wastewater and they obtained a retention of >70% protein and >90% lipids. In our study a much higher protein retention was obtained (>98%) even though the MWCO in the present work is expected to be > 200 kDa.

Investigation of the organic load in the fractions before and after PF and UF, using the water quality indicators COD, BOD₅, TSS and salt (Table 4), shows that the retention of COD was about 12% and 42% for TSP and SC, respectively. In respect to TSP, the COD level was almost unaltered after PF, whereas the PF retained almost 28% of the COD in the case of SC. The TSS level was reduced with more than 95% in both of the UF-PERs. A large part of the TSS was already retained by PF (approx. 51% and 22% for TSP and SC, respectively). There was no significant (P < 0.05) change in the BOD₅ level in any of the outlets for either TSP or SC, indicating that PF and UF are not affecting the biological matter in these brines. This might be due to the fact that smaller biomolecules, affecting BOD₅, are perfectly capable of permeating the 40 nm pore size of the ceramic membrane. Indeed, the BOD₅ is linked to the ability of biological organisms to break down organic material and since the brines contain compounds that are complex/difficult to break down this may explain the resilience of BOD₅. Cassini et al. (2010) studied ceramic UF of wastewater from isolated soy protein production. They reported retention of 26% and 85% of COD and TSS, respectively, which are data comparable to our results. In contrast to our results, a retention of about 80% BOD₅ was achieved by Mameri et al. (1996) who studied fishery washing water. Overall, the values found for COD, BOD₅ and TSS in TSP and SC were all higher than the general levels reported for all types of herring processing.

The content of trace elements, i.e. Zn, Fe, Mg, Ca, P and N is presented in Table 5, from which it is seen that there was no significant (P < 0.05) difference in the concentration of zinc in the RAW BRINE, UF-PER and UF-RET for either of the two brines. Iron, on the other hand, was retained by 85% and 91% in SC and TSP, respectively. Magnesium was present at a significantly (P < 0.05) higher level in SC compared to TSP, with 533 μg/g and 307 μg/g in the RAW BRINES, respectively, and the concentrations were unchanged during PF and UF treatments. The concentration of calcium was remarkable, as it was slightly increased (yet not significantly) from 133 to 160 μg/g in the UF-PER in the case of SC. However, it was significantly (P < 0.05) retained in the case of TSP (265–160 μg/g). To the best of our knowledge, the retention of Zn, Fe, Mg and Ca in UF treated herring brines has not been reported previously. The retention of some of the trace element may be due to complexation to protein.

The content of phosphorus in TSP and SC RAW BRINES was high; 2875 and 1880 mg/L, respectively (Table 5), and even higher in the corresponding UF-PERs; 3228 and 2188 mg/L, respectively. The concentration of phosphorous was higher in permeate compared to the inlet i.e. the retention was negative. Negative retention has previously been observed in UF studies i.e. when charged macromolecules are retained by the membrane, negative retention can be observed for the co-ions (Akred, Fane, & Friend, 1980; Radiman, Keteri, & Doo Sue, 2002). Hence, the data in this study indicate that the retained macromolecules are negatively charged and the phosphorous retention is negative due to electrostatic repulsion by the negatively charged macromolecules.

PF and UF retained 59% and 44% nitrogen in SC and TSP, respectively, resulting in 0.35% and 0.81% nitrogen in the UF-PERs. Szymczak and Kolakowski (2012) studied the losses of nitrogen from herring to brine during marination. They found that herring meat contain 2.6–2.9% nitrogen of which 18–27% was lost during marination, thus values of 0.5–0.8% were expected to be found in the RAW BRINES. They observed that a higher nitrogen loss was obtained from fillets than from carcasses. In this study a significantly (P < 0.05) higher nitrogen content was found in TSP than in SC (1.44% vs. 0.86%) indicating that the carcasses might lose more nitrogen than the fillets and also that ripening time has a profound effect on nitrogen loss. This might be explained by the fact that even though TSP is a result of marination of carcasses, the herrings are subsequently placed back in the brine as fillets and thus not solely exposed in the brine as carcasses.

3.3. Recovery of high value biomolecules

The main goal of this work is to recover high value biomolecules from spice brines into HMW and LMW fractions. Due to the high market value of fish proteins and lipids and the increasing interest in natural antioxidants, the marinated herring brines can represent an untapped source of valuable biomolecules. Therefore, protein, fatty acids (FA), n-3-fatty acid (n-3-FA) and TPC were determined. The content of protein, FA, n-3-FA and TPC in TSP RAW BRINE were 65.1 mg/mL, 5.9 mg/mL, 1.1 mg/mL and 3.7 mg GAE/mL, respectively. In TSP UF-PER, these concentrations had decreased to 20.1 mg/mL, 0.05 mg/mL, 0.0 mg/mL and 3.8 mg GAE/mL, respectively. As seen in Fig. 2, the retention was close to 80% for protein, 100% for both FA and n-3-FA for TSP, whereas the TPC was not significantly (P < 0.05) retained by the membrane. SC RAW BRINE and UF-PER had protein contents of 45.3 mg/mL and 10.5 mg/mL, respectively. The FA and n-3-FA were reduced in the SC RAW BRINE and UF-PER from 5.0 mg/mL, 0.05 mg/mL and from 0.68 mg/mL to 0.0 mg/mL, respectively. The retention of protein, FA and n-3-FA was similar for SC and TSP. However, comparing the partitioning of phenolics in SC vs. TSP, there was a clear difference. In SC RAW BRINE there was 2.58 mg GAE/mL which decreased to 1.58 mg GAE/
mL in the UF-PER, i.e. the membranes had retained almost 40% phenolics. In TSp, no phenolics were retained and it is unclear what causes this difference between SC and TSp. It can be speculated that the phenolic compounds present in TSp are smaller in size due to a longer ripening time and might therefore pass through the membrane compared to the phenolics in SC which might be bigger and therefore retained. The retention of phenolics by ultrafiltration has been studied by Galanakis, Markouli, and Gekas (2013) with three different MWCOs (100, 20 and 1 kDa) and two different dilutions of winery sludge (containing 0.48 and 1.97 mg phenols/mL). The retention was 56–85%, which might be explained by the lower MWCO values used in their work compared to ours.

To determine if oxidation was exacerbated under UF conditions, TBARS-value of PERs and RETs were determined for TSp and SC. From Fig. 3 a notable difference between the two brines is seen which could be explained by the longer ripening time of TSp compared to SC. TSp RAW BRINE had 45 nmol MDAeq/mL, which decreased to 29 nmol MDAeq/mL in the UF-PER and increased to almost 70 nmol MDAeq/mL in the UF-RET. In contrast, SC RAW

### Table 4
COD (g/L), BOD₅ (g/L), TSS (g/L) and salt content (wt%) in RAW BRINE, PF-PER and UF-PER and UF-RET after consecutive PF and UF treatment of TSp and SC.

<table>
<thead>
<tr>
<th></th>
<th>RAW BRINE</th>
<th>PF-PER</th>
<th>UF-PER</th>
<th>UF-RET</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSp COD (g/L)</td>
<td>124 ± 2.9²</td>
<td>128 ± 3.4²</td>
<td>109 ± 2.5²</td>
<td>121 ± 2.5²</td>
</tr>
<tr>
<td>TSp BOD₅ (g/L)</td>
<td>23 ± 0.3³</td>
<td>23 ± 0.9³</td>
<td>23 ± 0.3³</td>
<td>23 ± 0.3³</td>
</tr>
<tr>
<td>TSp TSS (g/L)</td>
<td>16 ± 1.8⁴</td>
<td>8 ± 2.1⁴</td>
<td>0.3 ± 0.1⁴</td>
<td>7 ± 1.3⁴</td>
</tr>
<tr>
<td>TSp Salt (wt%)</td>
<td>13.4 ± 0.1⁵</td>
<td>13.2 ± 0.1⁵</td>
<td>13.5 ± 0.2⁶</td>
<td>13.1 ± 0.1⁵</td>
</tr>
</tbody>
</table>

Values are given as mean (n = 2 for COD and BOD₅, n = 3 for TSS and salt) ± standard deviation (absolute value). By rows, same letter indicate no statistically difference (P < 0.05).

### Table 5
Zn (μg/g), Fe (μg/g), Mg (μg/g), Ca (μg/g), P (mg/L) and N (mg/g), in RAW BRINE, UF-PER and UF-RET after consecutive PF and UF treatment of TSp and SC.

<table>
<thead>
<tr>
<th></th>
<th>RAW BRINE</th>
<th>UF-PER</th>
<th>UF-RET</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSp Zn (μg/g)</td>
<td>7.19 ± 0.17²</td>
<td>6.06 ± 0.68²</td>
<td>9.31 ± 1.50²</td>
</tr>
<tr>
<td>TSp Fe (μg/g)</td>
<td>6.79 ± 0.33³</td>
<td>0.61 ± 0.05³</td>
<td>4.44 ± 0.05³</td>
</tr>
<tr>
<td>TSp Mg (μg/g)</td>
<td>307 ± 0.81³</td>
<td>277 ± 2.12³</td>
<td>299 ± 2.68³</td>
</tr>
<tr>
<td>TSp Ca (μg/g)</td>
<td>265 ± 8.83³</td>
<td>160 ± 4.56³</td>
<td>243 ± 7.52³</td>
</tr>
<tr>
<td>TSp P (mg/L)</td>
<td>2875 ± 5³</td>
<td>3228 ± 19.0³</td>
<td>3723 ± 73³</td>
</tr>
<tr>
<td>TSp N (mg/g)</td>
<td>14.4 ± 0.16³</td>
<td>8.10 ± 0.01³</td>
<td>13.8 ± 0.06³</td>
</tr>
</tbody>
</table>

Values are given as mean (n = 2 for Zn, Fe, Mg, Ca and N, n = 3 for P) ± standard deviation (absolute value). By rows, same letter indicate no statistically difference (P < 0.05).
BRINE had only 5.4 nmol MDAeq/mL which was unchanged in the UF-PER and UF-RET. These results indicate that, regarding oxidation, SC was stable during the PF and UF, whereas significant (P < 0.05) oxidation took place in the case of TSP, which was significantly (P < 0.05) more oxidized than the SC. The brines have different initial compositions and the retention of phenolics was different for the different brines, which may explain the observed results.

This study has tested the EF and PF in combination with UF for the recovery of high value biomolecules, i.e. protein, FA, n-3-FA and phenolics into retentates and permeates. The consecutive treatment with PF and UF reduced the COD, TSS, Fe and N content in the spice brines. The generated permeates were fat-free and contained a phenolic content of up to 3.8 mg GAE/mL as well as a notable phosphorous content. In contrast, the retentates were loaded with proteins and lipids. This work indicates that ceramic UF can recover biomolecules from the spice brines generated during the marinated herring production although further optimization is needed in order to improve the separation of the HMW and LMW biomolecules.

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References


