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Membrane fractionation of herring marinade for separation and recovery of fats, proteins, amino acids, salt, acetic acid and water

Lene Fjerbæk Søtoft *, Juncal Martin Lizarazu, Behnaz Razi Parjikolaei, Henrik Karring, Knud V. Christensen

University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark

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ABSTRACT

In the production of marinated herring, nearly one ton of acidic saline marinade is produced per 1.5 tons herring fillet. This spent marinade contains highly valuable compounds such as proteins and amino acids. Membranes are suited to recover these substances. In this work, six membrane stages are employed: microfiltration (MF) (0.2 μm), ultrafiltration (UF) (50, 20, 10 and 1 kDa) and nanofiltration (NF).

The most promising stages are 50 kDa UF and NF based on SDS–PAGE analyses and total amino acid concentration. The 50 kDa stage produces a protein concentrate (>17 kDa). NF produces a retentate containing sugars, amino acids and smaller peptides and a NF permeate containing salt and acetic acid ready for reuse. 42% of the spent marinade is recovered to substitute fresh water and chemicals. The waste water amount is reduced 62.5%. Proteins are concentrated 30 times, while amino acids and smaller peptides are concentrated 11 times.

1. Introduction

The seafood industry for human foods is a very water-intensive industry (Afonso and Borquez, 2002; Almas, 1985; Matthiasson and Sivik, 1978). The waste water is generally characterized by a high organic load and a varying salt content (Vandanjon et al., 2002). At the same time, there is a huge potential for recovery of valuable compounds of marine origin and make-up water from the waste fractions. This is important for the (1) better utilization of valuable marine compounds and new value-added by-products, (2) reduction in raw material consumption for improved process economy and (3) reduction of the environmental impact of food production.

A technology which can reduce water consumption in water-intensive industries is membrane separation (Afonso and Borquez, 2002; Almas, 1985; Matthiasson and Sivik, 1978). Additionally, membrane technology can offer separation/recovery of particles and molecules in very specific ranges making it very interesting for byproduct separation.

Microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and electrodialysis are already seen as established technologies as discussed by Galanakis (2012), but are sensitive to fouling due to the nature of the raw material (Galanakis, 2012). Several studies have reported the use of MF, UF, NF and reverse osmosis (RO) for separation, removal or recovery of organic material from fish industry waste water streams (Dumay et al., 2008; Ferjani et al., 2005; Matthiasson and Sivik, 1978; Li et al., 2006, 2008; Perez-Galvez et al., 2011; Stine et al., 2012; Vandanjon et al., 2002, 2009).

Because membrane processes are carried out at a relative low temperature, they offer an improved preservation of the concentrated compounds such as proteins compared to traditional thermal or chemical processes (Dumay et al., 2008). Matthiasson and Sivik (1978) were the first to demonstrate the usefulness of UF and RO for processing various waste waters from herring processing including spent herring marinade with 15–22 wt% salt. The aim of that study was to recover a protein concentrate and reduce the organic load in the waste water. The waste water COD (Chemical Oxygen Demand) load was reduced by up to 97% and protein was concentrated up to 10 wt% (Matthiasson and Sivik, 1978). These authors suggested combining membrane filtration with evaporation. Membrane filtration would then remove 65–90% of the water and make a 15–20 wt% commercial protein concentrate, which then by evaporation could be processed into 30–40 wt% protein (Matthiasson and Sivik, 1978).

Ceramic NF membranes (1 kDa) were used by Afonso and Borquez (2003) to concentrate protein from fish meal waste water. An economical assessment of a 10 m^3/h fish meal waste water treatment plant showed a rate of return of 17% and a feasible process (Afonso et al., 2004). Since then, there has been a huge development within membrane processes, an increased interest in value-added marine products and a rise in environmental awareness.

* Corresponding author. Tel.: +45 6550 7479.
E-mail address: lfj@kbm.sdu.dk (L. Fjerbæk Søtoft).

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It is expected that membrane fractionation of e.g. protein hydrolysates is not characterized by a sharp cut-off. Bourseau et al. (2009) observed this in the fractions obtained after a UF (4000 Da cut-off) and NF (300 Da cut-off) separation sequence of two fish protein hydrolysates. Size-exclusion chromatography showed how larger molecules were retained in the retentate and the smaller molecules were present in the permeate, but with a mixed retention in the intermediate molecular weight (MW) region (Bourseau et al., 2009). Beaulieu et al. (2009) fractionated a herring hydrolysate with similar results. A 50 kDa retentate had the highest total amino acid content (74% dry matter), while RO retentate contained most minerals (42% dry weight). The used stages were 0.3 μm, 50 kDa, 10 kDa, 1 kDa, 200 Da (NF) and <200 Da (RO).

The observed MW cut-off for a specific process is a combination of the chemical and filtration properties of the membrane, secondary layer(s) and feed, which can cause differences between nominal and observed cut-offs. Rejection of bovine serum albumin by a 150 kDa ceramic membrane has been found to depend on pH and whether or not 10 wt% NaCl is in the model solution. With 10 wt% NaCl and pH 9.0, the lowest rejection of 96.5% is obtained while at lower pH (4.8 and 6.8) rejection is >99% also when NaCl is present (Kuca and Szaniawska, 2009).

The presence of salts can reduce the value of protein or amino acids concentrate, but diafiltration can be used to purify the concentrates even more. Diafiltration is a membrane process where new solvent is added to an existing concentrate. The smaller permeating molecules such as sugars or salts are then washed away during a filtration as permeate, while the largest molecules for instance proteins are retained and purified by the membrane. This can be carried out as a continuous or batch process and can be a way to remove smaller molecules from a retentate, when a higher concentration of the largest retained molecules is of interest as done by Taheri et al. (2014) on herring brine.

As organisms of marine origin are adapted to an environment very different from the terrestrial, they produce numerous interesting compounds such as pigments, proteins, polysaccharides and lipids. Additionally, the market and interest in marine nutraceuticals are growing significantly (Rasmussen and Morrissey, 2007). Examples could be marine proteases (Bougatef, 2014) and other bioactive peptides (Picot et al., 2010).

The main aim of the present study is to recover valuable fractions from a fish industry waste and characterize the properties and potential use of each fraction. Additionally, the purpose is to reduce the amounts of saline waste water with high organic content discharged from the plant. This work is unique in the number of product fractions (six consecutive membrane stages) and the scale of the experiments with a starting volume of 120 L of spent herring marinade. The multiple objectives of this work can be specified as (1) recovery of organic particles, (2) recovery of proteins and amino acids, (3) recovery of fats, (4) recovery of sugars, (5) recovery of water and chemicals of sufficient quality for reuse and (6) volume reduction of waste for further treatment.

2. Material and methods

2.1. Materials

A fresh sample of spent herring marinade (~120 L) was provided by the Danish fish food producer LAUNIS Fiskekonserves A/S. The sample was kept at 3–7 °C. Before addition of the herring, the marinade is composed of water, acetic acid and salt. Thus, fish meat residues, fat, proteins, peptides and free amino acids can be expected to be present in the marinade in addition to acetic acid and salt after marinating the herring under anaerobic conditions and subsequent removal of the fillets.

An inspection of the marinade showed visible fish meat residues and a fat layer. This had to be removed prior to any filtration to protect the membranes. Due to the low temperature, the fat solidifies on top of the liquid and can be removed efficiently by skimming (no visual oil droplets left).

Chemicals (acetonitrile: HiPerSolv Chromanorm from VWR, PROLAB and water) for HPLC were of UV-grade, whereas cleaning chemicals for the membrane setups (citric acid and NaOH, VWR) were of food grade quality. Water used for membrane cleaning was ion-exchange quality (conductivity below 10 $\mu S\,cm^{-1}$).

2.2. Methods

2.2.1. Sieving and membrane filtrations

A series of membrane filtrations with various pore sizes has been carried out. Sieving is used as pretreatment and has been done as manual batch sieving with dead-end sieves. Their characteristics can be seen in Table 1.

The cross flow membrane unit used is a Labstack M20 for flat-sheet membranes (Alfa Laval). The retentate is recycled to the feed tank in order to concentrate the retentate. The setup is equipped with a feed pump (Hydracell), an inline feed heat exchanger, two feed side pressure gauges and a pressure control valve on the retentate side. Feed flow rate was controlled by adjustment of the pump speed. Permeate is collected separately during filtrations. A weight (Dansk Vægt Industry A/S, 0–35 kg) was used to measure the permeate flow rate. Temperature was controlled by connecting a cooling unit (Heto HMT 200) to the heat exchanger. Additionally, the feed tank was cooled by insertion into a cooling tank during NF to reduce the temperature increase during operation (Fig. 1). During each run, only one type of membrane was used at a time. The fractions and samples are cooled immediately at 3–7 °C after a filtration. The membranes used in each run can be seen in Table 2 specified with either pore size, molecular weight cut-off (MWCO) or rejection.

The volume reduction (VR) for a specific fraction is calculated as:

$$VR = \text{Concentrate volume}/\text{Initial volume} \quad (1)$$

2.2.2. Dry matter and ash content

Dry matter (DM) and ash content measurements were performed at 105 °C and 550 °C, respectively. The analyses were done in triplicate according to DS 204:1980.

2.2.3. Protein quantification by absorbance at 280 nm

Total protein concentration in the different marinade fractions was determined by measuring the absorbance at 280 nm (Beaven and Holiday, 1952; Layne, 1957). For unknown complex protein mixtures, it is commonly accepted that 1 absorbance unit at 280 nm equals 1 mg/mL protein (light path length of 1 cm). The fractions were diluted in pure marinade solution (9.0 wt% NaCl, 2.0 wt% acidic acid, pH 4.15) to obtain absorbance values in the range 0.1–0.7. Subsequently, absorbance at 280 nm (A280) was measured for 1 mL samples using the pure marinade solution as reference. Dilutions and A280 measurements were performed in triplicates. Finally, the measurements were corrected for the dilution factor to obtain absolute A280 values for the fractions.

Table 1
Sieves used for pretreatment of the herring marinade.

Sieve area (mm)	Material	Supplier	Mesh size (mm)
200 × 50	Stainless steel	Retsch	0.5
200 × 25	Stainless steel	Retsch	0.18
201 × 25	Stainless steel	Retsch	0.145
202 × 25	Stainless steel	Retsch	0.045