Fractionation of herring marinade with membrane technology

Fjerbæk Søtoft, Lene; Martin, Juncal; Razi Parjikolaei, Behnaz; Karring, Henrik ; Christensen, Knud Villy

Published in:
14th Nordic Filtration Symposium

Publication date:
2012

Document version:
Accepted manuscript

Document license:
Unspecified

Citation for pulished version (APA):
Fjerbæk Søtoft, L., Martin, J., Razi Parjikolaei, B., Karring, H., & Christensen, K. V. (2012). Fractionation of herring marinade with membrane technology. *14th Nordic Filtration Symposium*, *1*(1).

FRACTIONATION OF HERRING MARINADE WITH MEMBRANE TECHNOLOGY

Lene Fjerbæk Søtoft*, Juncal Martin, Behnaz Razi Perjikolaei, Henrik Karring, Knud V. Christensen.
University of Southern Denmark
Campusvej 55, 5230 Odense M
Contact information: email: lfj@kbm.sdu.dk; phone no.: +45 6550 7479

Abstract (200 words)
Reuse of waste products from the food industry is of great interest worldwide. Seafood industry is generally considered to be a water-intensive industry producing a waste water with a high organic load and a high salt content. This work presents experimental work using sieving, micro-, ultra- and nanofiltration as basis for a possible process flow in which fats, protein fractions, peptide and sugar & salt fractions are produced from used herring curing marinade. These fractions might form the basis for a value-added production from and reuse of the waste water. The work further shows that the use of RO for reduction of the waste water volume is not feasible due to the inherent high salt and sugar concentration of herring marinades leading to an osmotic pressure of the waste water of around 70 bar.

Keywords: Microfiltration, ultrafiltration, nanofiltration, protein fractionation, herring marinade

Introduction

Reuse of waste products from the food industry is of great interest worldwide. It is important for several reasons: An increasing human population, a reduction in the environmental impact from food production and an increased profit by reduction of raw material consumption and value-added products from waste.
Membrane technology offers promising novel waste product fractionation for potential value-added products and improved reuse potential of i.e. chemicals and water. Optimized fractionation combined with new market areas can also lead to value-added products with novel properties and sales potential. This work presents results on fractionation of an acidic herring marinade from an industrial producer and characterization of properties and potential use of each fraction.
Seafood industry is generally considered to be a water-intensive industry producing a waste water with a high organic load and a varying salt content [1]. Membrane technologies such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis have been extensively reported for removal of organic material from fish industry water streams [1-6]. Work started in the early 1980'es [7] and is still ongoing. Investigators have been testing both ceramic and polymeric membranes.
The purposes are a.o.:

- Reduction of organic load in waste water (COD removal)
- Reduction of waste water amount and recovery of water for reuse
- Recovery of proteins for existing products or novel usages
- Recovery of bioactive compounds

When suggesting reuse to a relevant company, it is important to suggest a procedure that can comply with the HACCP programme of the company including an integrated plan to control

the quality and safety during reuse. An example has been reported by Casani et al. (2006) [8]. The complex composition of the water stream can also affect the outcome of the membrane treatment processes. Presence of salt can i.e. change the rejection of the proteins as observed by Kuca and Szaniawska (2009). They report that for ceramic membranes protein rejection decrease when NaCl is present and with increasing pH [9].

The aim of the study is to separate a waste product, used herring marinade from LAUNIS Fiskekonserves A/S, into value-added fractions with novel properties with increased sales or reuse potential. Many membrane filtration steps were included to obtain a large number of fractions for further study. The results presented in this paper are based on analysis on the collected summed fractions of each filtration.

Prior investigations of the used marinade yielded ~10% dry matter, SDS-PAGE[1] analysis of > 6 kDa proteins and a MALDI-TOF[2] for peptides < 3 kDa (mainly below 2 kDa). Initially, the marinade consists of acetic acid, salt and water before addition of the herring. The SDS-page and MALDI-TOF analysis of the herring marinade after storage of the herring was performed in order to get some size ranges of the proteins in the marinade. The most concentrated ranges were 6 -18 kDa, 20 -28 kDa, 35 - 62 kDa and 70 -100 kDa. The MALDI-TOF showed several sharp peaks interpreted as stemming from peptides. This knowledge was used to setup the treatment steps, as i.e. the MWCOs of the UF membranes was decided upon based on these results.

Materials & Methods

Materials

A fresh sample of used marinade was provided by LAUNIS Fiskekonserves A/S in 4 plastic buckets (around 30 liters of sample each). The sample was kept at 3-7 ºC in a cooling tank. Initially, the marinade composes of water, acetic acid and salt before addition of the herring. After marinating the herring and subsequent removal of the fillets, meat residues, fat, proteins, peptides and amino acids can be expected to be present in the marinade.

Visual inspection showed that the sample contained fish meat residues and a fat layer, which had to be removed before the beginning of the filtration process. 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). The marinade was then sieved consecutively through four mesh sizes in order to remove meat and larger visible particles (see table I).

Table I: Sieves used for pretreatment of the herring marinade.

Sieve area [mm]	Material	Supplier	Mesh size [mm]
200 x50	Stainless steel	Retsch	0.5
200 x 25	Stainless steel	Retsch	0.18
201 x 25	Stainless steel	Retsch	0.145
202 x 25	Stainless steel	Retsch	0.045

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

[1] Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
[2] Matrix-assisted laser desorption/ionization - time-of-flight mass spectrometry

NaOH, VWR) were of food grade quality. Water used for membrane cleaning was ion-exchange quality (conductivity below10 $\mu S \cdot cm^{-1}$). The used membranes will be described below.

Methods

Membrane setup

The cross flow membrane unit used was a Labstack M20 from Alfa Laval, applying flat-sheet membranes. During a run the retentate was recycled to the feed tank in order to concentrate the retentate. The setup was 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. A weight (Dansk Vægt Industry A/S, 0-35 kg) was used to measure the permeate flow rate. Temperature was controlled by connection a cooling unit (Heto HMT 200) to the heat exchanger. Additionally, the feed tank was cooled by insertion into a cooling tank during NF and RO to reduce the temperature increase during operation. During each run only one type of membrane (MWCO) was used at a time. The membranes used in each run can be seen in Table II.

Table II: Flat sheet membranes used for herring marinade fractionation

Process	Membrane type	Material	Pore size/MWCO /Rejection
MF	Alfa Laval- MFP2	Fluoro polymer	0.2 µm
UF50	DSS-GR51PP	Polysulphone	50 kDa
UF20	Alfa Laval-FS61PP	Fluoro polymer	20 kDa
UF10	DSS-ETNA10PP	Composite fluoro polymer	10 kDa
UF09	Alfa Laval-ETNA01PP	Fluoro polymer	1 kDa
NF	Alfa Laval-NF	Polyamide on polyester	Reject > 98% $MgSO_4$ (2000 ppm, 9 bar, 25ºC)
RO	RO98pHt	Composite on polypropylene	Reject > 97% NaCl (2000 ppm, 16 bar, 25ºC)

Dry matter, ash content and absorbance test

Dry matter and ash analysis was performed following European Standards. The analysis' were done in triplicate. The absorbance of the liquid streams was measured on a spectrophotometer at $\lambda = 280$ nm, where at low absorbance the content of aromatic amino acids is linear proportional to the absorbance. As a rule of thumb protein mixture concentrations [mg/mL] at this wavelength are equal to absorbance divided by path length [1 cm].

HPLC

Analysis for ethanol, glycerol, fructose, glucose, lactose and sucrose were done by isocratic HPLC (Agilent series 1100) performed using a Luna 5µm NH2 100Å 250x4.60 mm column with 65:35 (vol%) acetonitrile:H_2O as eluent. The column temperature was 25 °C, injected sample size 5 µL, the eluent flow 0.5 mL/min and analysis time 20 min. The analysis' were done in duplicate.

Experimental work

The liquid waste product contains fats, proteins, small organic acids, salts and sugar. Based on the initial analysis and knowledge of the waste product, a fractionation procedure was established to separate the components into 9 fractions based on protein seize, peptide, sugar

and salt content. Then, several membrane filtrations with decreasing MWCO were carried out in series on the same 113 liter batch of liquid waste.
As seen from table II and figure 1, the total membrane sequence covered the range of micro-, ultra-, nanofiltration to reverse osmosis, so fractions of fats, suspended solids, proteins (more than one), sugars and salts are obtained.

Results & Discussion
Membrane fractionation of the liquid waste product is possible. The acidic herring marinade was separated into fractions of potential value or reuse. Selected results and analysis' are presented below.
Removal of fats and sieving were done as pretreatment steps to make the membrane filtrations run in the best possible way. Filtrations were operated as constant pressure filtrations.
A typical filtration curve with flux as a function of time can be seen in Figure 2. Due to the large quantities of marinade, the process was carried out in three steps, where fresh marinade was supplied between stage 1 and 2 and cleaning was performed between stage 2 and 3.

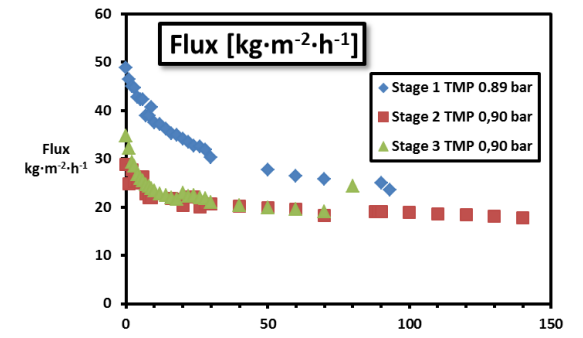

Figure 2: Microfiltration of herring marinade with 0.2 µm membrane. Permeate flux as function of time. Feed flow rate = 5kg/min, temp. 20-24°C transmembrane pressure between 0.89 and 0.91 bar.

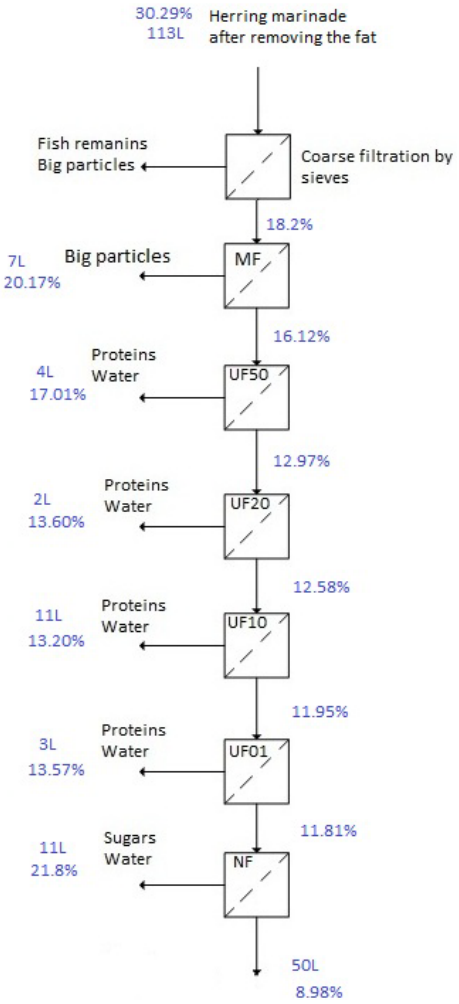

Figure 1: Mass balance and dry matter content [wt%] for herring marinade during membrane fractionation.

A rapid initial flux decline can be observed, also after cleaning, but also a steadier, slower decline during the whole period of time can be seen. Initial flux was ~50 L/m²/h and declined to 18 L/m²/h.
This picture of flux decline during a filtration is typical for the observations during the residual filtrations (results not shown). A mass balance of the filtrations with the collected concentrate fractions can be seen in Figure 1.
The last successful separation was NF at 26 bar, where 50 L of clear permeate was collected. RO was also tested, but due to the high salt concentration the osmotic pressure of the feed exceeds the possible operational pressure of the membranes. The ash content was analyzed to 7.96 wt% corresponding to an osmotic pressure of ~70 bar.

Analysis of dry matter and ash content of the collected fractions can be seen in Figure 3.

Lene Fjerbæk Søtoft
Institute of Chemical Engineering, Biotechnology and Environmental Technology
University of Southern Denmark

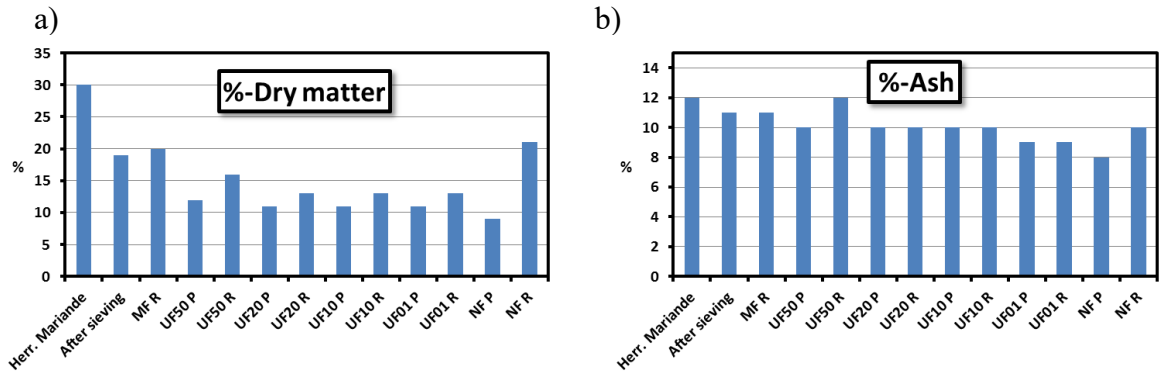

Figure 3: Dry matter content a) and ash content % b) of fresh marinade and collected permeate (P) and retentate (R) samples during processing.

As seen, the dry matter content decreases after sieving due to removed larger organic matter, while inorganic matter (ash%), primarily sodium chloride salt, passes through the sieves and membranes unhindered as expected. The increase in dry matter in the retentates must therefore be due to retained organic matter. As seen from figure 4, the absorbance at $\lambda = 280$ nm increases which indicates an increase in protein concentration as should be expected. The rejection is relatively low on the individual UF-membranes which based on the initial SDS-PAGE analysis is not surprising and indicates that a fractionation into different protein fractions based on molecular weight has taken place. The large increase in rejection on the NF-membrane with only a small increase in ash content indicates a high content of peptides based on the absorbance and perhaps sugars based on the dry matter content (Figure 3a).

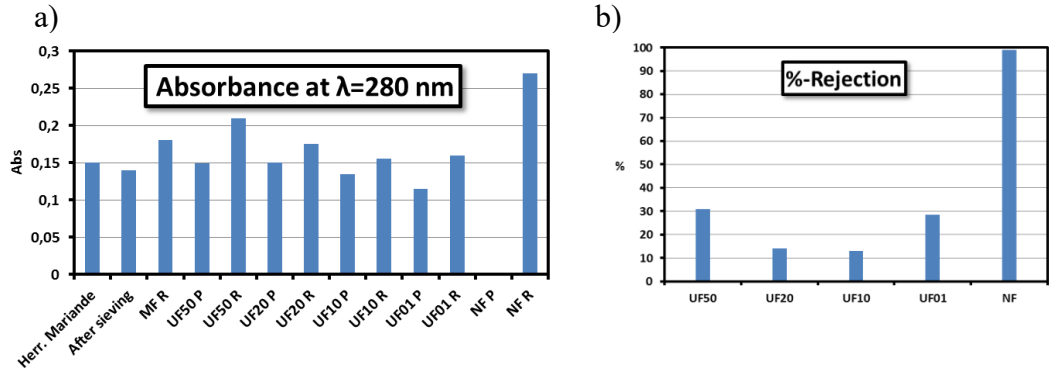

Figure 4: Absorbance of collected fraction from UF50 to NF at $\lambda = 280$ nm a) and calculated rejection based on absorbance in concentrate and permeate b).

The HPLC analysis of the NF permeate and retentate indicates that sugars permeate the membrane while peptides are retained. Further, glycerol is found to be present in both permeate and retentate. The presence of glycerol is possibly due to acid-catalyzed hydrolysis of triglycerides (fats) into free fatty acids and glycerol during the herring curing process.

More analysis' are planned to elucidate more on the protein content of the fractions by SDS-page in comparison with the known MWCO of the membranes. The nature of the proteins and their potential as value-added products will be looked into to determine a final fractionation

Lene Fjerbæk Søtoft
Institute of Chemical Engineering, Biotechnology and Environmental Technology
University of Southern Denmark

process design. Additionally an economical evaluation will be carried out to investigate the potential in saving running costs, if some or more of the fractions are reused.

Conclusion

As seen, filtration and membrane separation is a possible process for fractionation of used herring marinade into concentrates of fats, protein fractions, a peptide and a salt & sugar fraction. Also it can be concluded, that due to the inherent high salt and sugar content in herring marinade a reduction in waste water volume using RO is not feasible.
Further, much work is still to be carried out in order to characterize the protein and peptide fractions obtained and purification of the protein fractions using diafiltration is to be tested.

Acknowledgements

The authors want to thank LAUNIS Fiskekonserves A/S, Industrivej Nord 2 – 4, 9982 Aalbæk, Denmark, for providing samples and insight into the herring curing industry, Danish Technological Institute, Kongsvang Allé 29, DK-8000 Aarhus C, Denmark, for sharing SDS-PAGE and MALDI-TOF analysis result, and the Danish Agency for Science Technology and Innovation for financing the Innovation Consortium *Natural Ingredients and Green Energy* of which this work is a part.

Reference List

[1] L. Vandanjon, S. Cros, P. Jaouen, F. Quemeneur, and P. Bourseau, Recovery by nanofiltration and reverse osmosis of marine flavours from seafood cooking waters, Desalination, 144 (2002) 379-385.
[2] M.D. Afonso and R. Borquez, Review of the treatment of seafood processing wastewaters and recovery of proteins therein by membrane separation processes - prospects of the ultrafiltration of wastewaters from the fish meal industry, Desalination, 142 (2002) 29-45.
[3] M.D. Afonso and R. Borquez, Nanofiltration of wastewaters from the fish meal industry, Desalination, 151 (2003) 131-138.
[4] M.D. Afonso, J. Ferrer, and R. Borquez, An economic assessment of proteins recovery from fish meal effluents by ultrafiltration, Trends in Food Science & Technology, 15 (2004) 506-512.
[5] R. Perez-Galvez, E.M. Guadix, J.P. Berge, and A. Guadix, Operation and cleaning of ceramic membranes for the filtration of fish press liquor, Journal of Membrane Science, 384 (2011) 142-148.
[6] J.J. Stine, L. Pedersen, S. Smiley, and P.J. Bechtel, Recovery and Utilization of Protein Derived from Surimi Wash-Water, Journal of Food Quality, 35 (2012) 43-50.
[7] K.A. Almas, Applications of Cross-Flow Membrane Technology in the Fishing Industry, Desalination, 53 (1985) 167-180.
[8] S. Casani, T. Leth, and S. Knochel, Water reuse in a shrimp processing line: Safety considerations using a HACCP approach, Food Control, 17 (2006) 540-550.
[9] M. Kuca and D. Szaniawska, Application of microfiltration and ceramic membranes for treatment of salted aqueous effluents from fish processing, Desalination, 241 (2009) 227-235.