clean technologies

MDPI

Article

Clean Technologies for Production of Valuable Fractions from Sardine Cooking Wastewaters: An Integrated Process of Flocculation and Reverse Osmosis

Maria João Pereira [1], Oceane Grosjean [2], Manuela Pintado [3], Carla Brazinha [1,*] and João Crespo [1]

[1] LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, FCT NOVA, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; mjl.pereira@fct.unl.pt (M.J.P.); jgc@fct.unl.pt (J.C.)
[2] Polytech Nantes, GBP Department, Nantes University, 44306 Nantes, France; oceane.grosjean@etu.univ-nantes.fr
[3] CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal; mpintado@ucp.pt
* Correspondence: c.brazinha@fct.unl.pt

Abstract: The increase in environmental consciousness and stricter regulations has motivated industries to seek sustainable technologies that allow valorising wastewaters, contributing to the profitability of overall processes. Canning industry effluents, namely sardine cooking wastewater, have a high organic matter load, containing proteins and lipids. Their untreated discharge has a negative environmental impact and an economic cost. This work aims to design an integrated process that creates value with the costly sardine cooking wastewater effluent. The research strategy followed evaluates coagulation/flocculation technologies as pre-treatment of the sardine cooking wastewater followed by reverse osmosis. Two different added-value products were obtained: a solid fraction rich in proteins, lipids (above 20%), and aromas that might be used for feed/pet/aquaculture applications and, from the processing of the resultant aqueous stream by reverse osmosis, a natural flavouring additive, which can be applied in food/feed. Additionally, the permeate from reverse osmosis presents a much lower organic load than the original raw material, which may be reused in the overall process (e.g., as water for washings) or discharged at a lower cost, with environmental benefits and economic savings.

Keywords: reverse osmosis; coagulation/flocculation; aroma recovery; sardine cooking wastewaters

check for updates

Citation: Pereira, M.J.; Grosjean, O.; Pintado, M.; Brazinha, C.; Crespo, J. Clean Technologies for Production of Valuable Fractions from Sardine Cooking Wastewaters: An Integrated Process of Flocculation and Reverse Osmosis. *Clean Technol.* **2022**, *4*, 276–295. https://doi.org/10.3390/cleantechnol4020016

Academic Editor: Sabino De Gisi

Received: 8 February 2022
Accepted: 11 April 2022
Published: 15 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Today, one of the world's major problems is waste management, a global challenge exacerbated by the amount and complexity of the domestic and industrial wastes generated. The focus of current European Union law is on zero-emission production processes, and the reduction of up to 50% agrofood wastes by 2030, which may be achieved by reducing pollutant discharge from industrial operations and the reuse and valorisation of wastes and effluents [1]. Another concern today is the massive use of water. Of these, the food and beverage industries are one of the largest consumers. The seafood processing industry plays a key role in water consumption, as 2% of all freshwater consumed in the food and beverage industry [2] is used throughout the processing steps, such as washing, cooking, freezing, disinfection and, floor cleaning.

In a very fragmented market, Portugal is the third-highest producer of canned seafood, representing 8% (74,133 tonnes) of the world production of canned sardines, from which the top 20 fish canning facilities produce around 44 thousand tonnes of canned products each year [3,4]. The canning industry generates a large amount of wastewater, which is particularly difficult to treat due to its high content of salts organic matter (lipids and proteins) [5,6].

To reduce the massive use of water and production of wastewater, while addressing an environmental pollution issue, several strategies need to be considered involving the processing of seafood wastewater by efficient, low-cost, and environmentally friendly technologies. First, it is important to identify the added-value molecules present in the wastewater to be valorised, by recovering those components for feed or food applications. Then, the remaining wastewater should be treated to reduce its organic load and obtain clean(er) water either to be reused in the overall process (the preferable option) or to be discharged. The European Directive 98/83/EC allows water reuse in the industrial process [7]. This approach brings further revenues to the seafood processing industries, both from the added-value food components [2] and from the reduction of the wastewater discharge costs.

Different methods can be used for the treatment of seafood wastewater, which can be physical methods: centrifugation, coagulation/flocculation, dissolved air flotation and activated carbon adsorption, membrane processes (microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and pervaporation), and biological treatments, as stand-alone or integrated systems [6,8].

Membrane separation processes may efficiently recover high-value, sensitive compounds, from seafood wastewater as these processes may operate under mild operating conditions, for example, at mild temperatures. In particular, reverse osmosis may also produce water from effluents, to be reused in the overall process and is suitable for processing seafood wastewater. Since it involves the use of dense membranes, it is not prone to intrapore fouling and may exhibit very high retention of small particles and solutes, including monovalent ions present in the water, allowing for the production of quality water that might be reused (depending on the feed concentration) [9,10].

Although the processing of wastewater by reverse osmosis may contribute to the profitability of the overall process, several issues should be addressed, due to the complexity of the wastewater. The most important issue is membrane fouling, due to the adsorption and accumulation of fouling compounds on the membrane surface, which consequently reduces efficiency and increases costs, ultimately compromising the efficiency of the process. For these reasons, reverse osmosis combined with different pre-treatments has been reported, including for the treatment of seafood wastewater [7,11,12].

Seafood wastewater may be processed by stand-alone coagulation–flocculation or by an integrated process of coagulation–flocculation, used as pre-treatment, followed by reverse osmosis.

In coagulation/flocculation operations, a coagulant agent is introduced to destabilise the interaction between organic compounds, by reducing repulsive forces between particles. The flocculant agent then binds the fine matter together for easier removal. The larger particles are then filtered and clear wastewater is obtained. The processing of seafood wastewaters by coagulation/flocculation for feed/food applications demands the use of food-grade coagulants and flocculants [8]. The natural polymer chitosan is a suitable coagulant [13] because, besides being harmless and biodegradable, it is a cationic polymer, so its molecules bind to the negatively charged surface of particles and colloids through ionic bonds or by hydrogen bridging. Flocculation is usually carried out with iron, aluminium salts, or other non-food grade chemicals, which indeed are efficient for cleaning the water, but would represent a limitation to this process, due to toxicity and health hazards posed by inorganic flocculants. To avoid this problem, some polysaccharides such as carrageenan, alginate, and carboxymethylcellulose can be used as food-grade coagulant/flocculant agents [14,15].

The treatment and valorisation of sardine cooking wastewater were selected as a case study in this work. The research strategy used considered the need for a pre-treatment of the raw material, because sardine cooking wastewaters are very heterogeneous and complex mixtures, with a high organic load. Therefore, a coagulation/flocculation pre-treatment was implemented and optimised. Then, the resultant aqueous stream was processed by reverse

osmosis, leading to a concentrate rich in aromas and an aqueous stream (the permeate) with a much lower organic load than the original raw material.

Some studies with the aim of valorising seafood cooking wastewaters were reported in the literature. Forghani et al. [2] recovered protein-enriched biomasses from shrimp boiling wastewaters by a combination of flocculation and dissolved air flotation, with a protein yield of 68–97%. Tremblay et al. [16,17] studied the concentration of aromatic fractions of snow crab cooking wastewaters by reverse osmosis, obtaining an aromatic concentrate with 92% of the total dry residues (formed by lipid, proteins, and minerals). When processing lobster cooking wastewaters by reverse osmosis, they obtained a fraction composed of minerals (60.4%), proteins (30.0%), and desirable aroma compounds.

The overall aim was to optimise the integrated process comprising coagulation/flocculation and reverse osmosis to valorise sardine cooking wastewaters obtaining two added-value products and, simultaneously, treating and reducing the discharge of the effluent with environmental and economic benefits. Following this approach is the aim to produce: (i) a solid fraction with maximised content in protein, lipids, and flavours/aromas, that results from the pre-treatment process, (ii) a reverse osmosis concentrate, with maximised content in flavours (namely achieving the highest possible concentration factor in reverse osmosis), and (iii) a reverse osmosis permeate with a minimised COD value (organic load).

2. Materials and Methods

2.1. Materials

The sardine cooking wastewater was kindly provided by A Poveira S.A. (Laúndos, Portugal). This effluent is the result of steaming the fish for 7 min at 100 °C.

For the coagulation–flocculation step, two different combinations were tested. Chitosan (HMW) from shrimp shells ≥75% (deacetylated) (Sigma-Aldrich, St.Louis, MO, USA) was used as a coagulant. As flocculants, λ-carrageenan from the cell walls of the red algae (Sigma-Aldrich, USA) and alginate from brown algae (Sigma-Aldrich, USA) were tested. All coagulant/flocculant compounds used were food grade.

The membrane used during the reverse osmosis studies was a commercial seawater reverse osmosis membrane FILMTECTM Flat Sheet SW30 HR (Dow Filmtec, Midland, MI, USA). A polyester support web (nominal pore size of 120 µm), a microporous polysulfone interlayer nominal pore size of (40 µm), and an ultra-thin polyamide barrier layer on the top surface (nominal pore size of 0.2 µm) comprised this thin-film composite membrane. The effective area of the membrane was 5.1×10^{-3} m^2.

2.2. Experimental Procedure

At the moment of sampling the sardine cooking wastewater, at the outlet of cooking chambers, skin acorn aqueous extract with antioxidant potential was added at 1% (v/v) concentration. The wastewater was collected, transported, and stored at −20 °C until processing. Figure 1 describes the flow of the experimental studies carried out in this work.

In order to produce valuable fractions from sardine cooking wastewater, coagulation/flocculation pre-treatment was applied before reverse osmosis.

Each processing step of the integrated process of coagulation–flocculation and reverse osmosis (see Figure 1) corresponds to different studies:

- In the **feed preparation** step, a study was performed encompassing the effect of adding the acorn extract to the sardine cooking wastewater on the chemical characteristics of the aroma profile (aiming for minimised oxidation of aromas, namely aldehydes, and minimised formation of sulphur compounds off-flavours). The feed preparation with the intended composition was selected.
- In the **coagulation–flocculation** step, studies were conducted aiming to determine the effect of the concentration of the coagulant and the effects of the type and concentration of the flocculant on the chemical composition of the supernatant and solid fraction (aiming at a clarification of the supernatant by maximised proteins′ and

lipids' recovery in the solid fraction). Twelve coagulant/flocculant combinations were evaluated.

- In the **reverse osmosis** step, studies were performed aiming to assess the effect of the feed source (aqueous fractions from each pre-treatment), when processed by reverse osmosis, on the composition of the concentrates (in terms of aromas) and of the permeates (in terms of COD, related to the organic load). The impact of the pre-treatment process on the membrane performance (membrane permeance) was also evaluated. For the four best coagulant/flocculant combinations, reverse osmosis experiments were performed for the selection of the combination of coagulant and flocculant concentrations, reaching the same final volumetric concentration factor of 3.

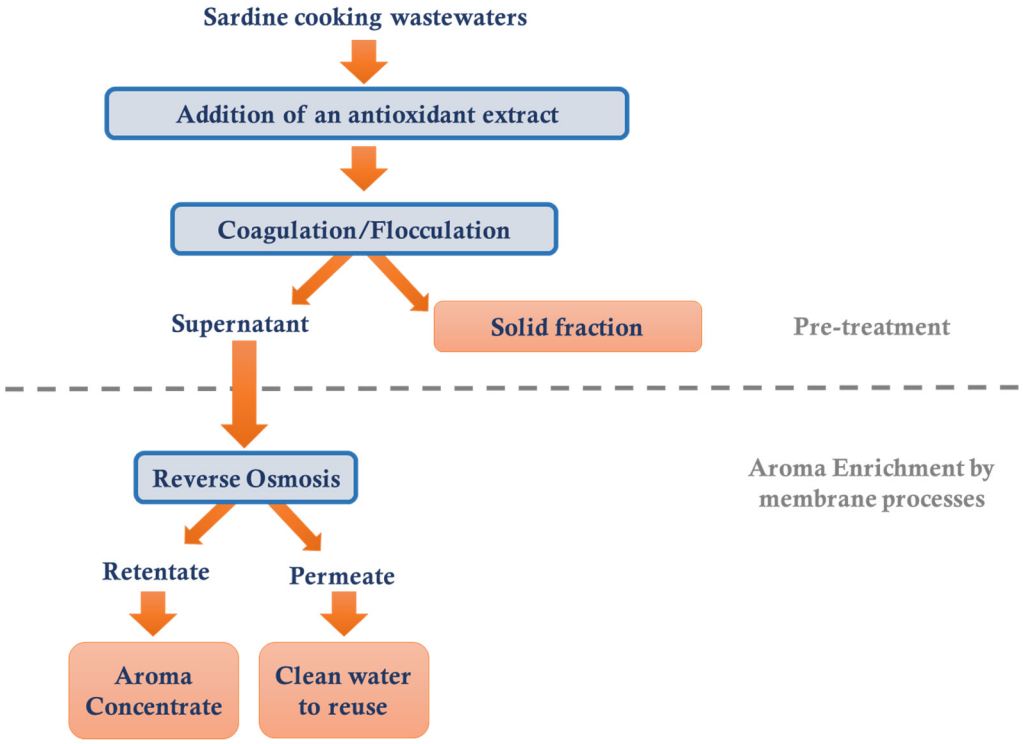

Figure 1. Flow diagram summarising the experimental studies performed in this work.

2.2.1. Coagulation/Flocculation Pre-Treatment

The pH of sardine cooking wastewater was adjusted to pH 4 to decrease the charge of organic matter, making it less water-soluble. Control tests were conducted with the three polysaccharides, applied as coagulant and flocculants, using chitosan at a concentration of 100 mg/L and carrageenan and alginate at 10 mg/L in order to compare with the situations where coagulant/flocculant combinations were tested. Two different combinations of coagulant and flocculant were tested, chitosan-carrageenan (Chi/CA) and chitosan-alginate (Chi/ALG). The different assays were performed in a 1.5 L jar using 1 L of the sample. The setup of this pre-treatment is explained in Figure 2. Chitosan was tested in a range from 100 to 600 mg/L. Both flocculants were tested in a range from 10 to 60 mg/L. The procedure for all samples was, after adding chitosan, high stirring for 3 min at 500 rpm at room temperature using a stirrer (OHS 100 digital, Velp Scientifica, Italy). In the last 10 s of high stirring, the flocculant agent was added and the stirring velocity was brought down to 40 rpm for 30 min. In the end, the sample was centrifugated (Marathon 22 KBR, Fischer Scientific, Waltham, Massachusetts, USA) at 3800× g for 20 min before removing the solid fraction from the supernatant. A sedimentation step was tested for both combinations (data not shown), however, centrifugation was found to be the best approach due to the longer operating time and larger installation area required by the sedimentation process.

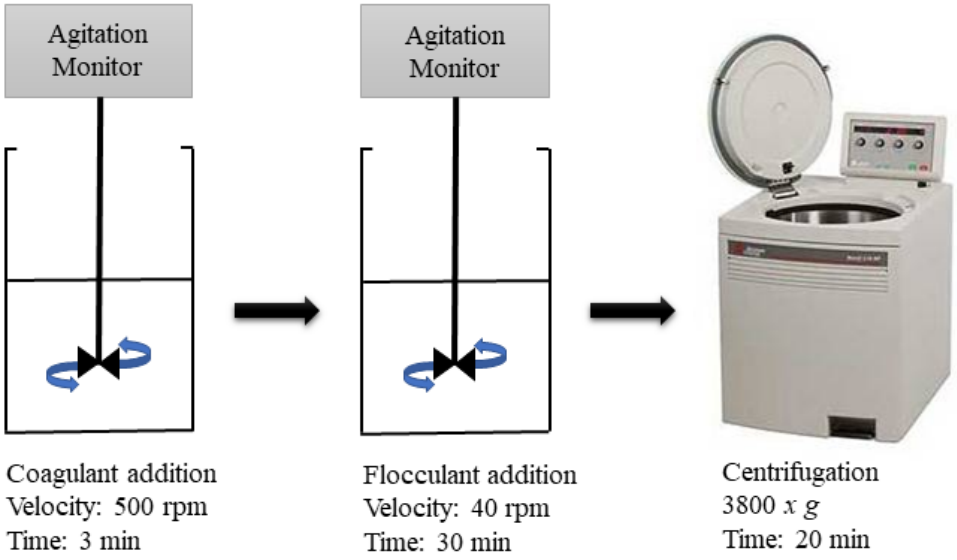

Figure 2. Schematic representation of the coagulation/flocculation process.

2.2.2. Reverse Osmosis

The reverse osmosis (RO) experiments for the selection of the best combination of coagulant and flocculant concentrations were performed in a laboratory test unit. In a cross-flow stainless steel test cell, a membrane area of 5.1×10^{-3} m^2 was used under controlled transmembrane pressure conditions (EVONIK METCell, Evonik, United Kingdom, see Figure 3). A porous stainless-steel disc supported the membrane in this test cell. The feed temperature was 40 °C, and the applied pressure was 40 bar, which was achieved with a MET pre-assembled argon gas unit. The feed reservoir was filled with 600 mL of the supernatant obtained after the coagulation–flocculation process.

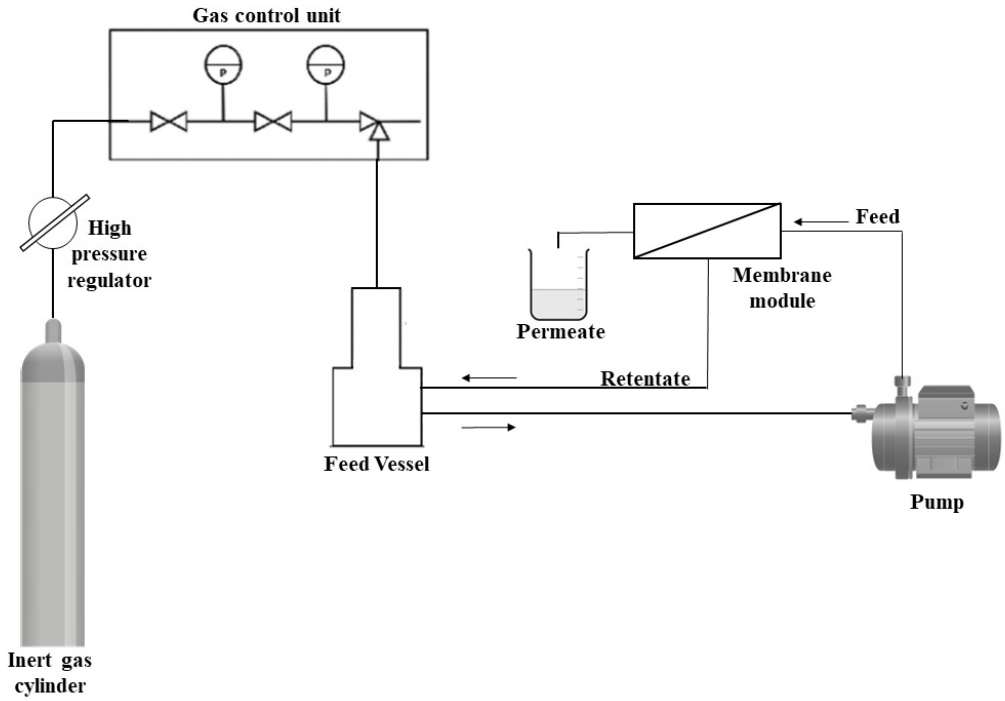

Figure 3. Experimental reverse osmosis setup using EVONIK METCell unit.

The protocol of the cleaning treatment, involving an alkaline step, was applied to restore the initial permeability operation. The cleaning sequence comprised an initial rinse of the membrane with water in total recirculation mode for 30 min, followed by alkaline treatment with 6 g/L NaOH in total recirculation mode for 30 min. After these stages, the membrane was rinsed with water until the neutralisation of the streams and the water flux was determined to evaluate the efficiency of the cleaning procedure. The membrane permeability after the cleaning procedure was restored (with at least 91% permeability recovery).

The permeate flux was determined throughout the filtration time by collecting instant permeate samples at different volumetric concentration factors. The permeate flux expressed in L/(m^2 h) was calculated by Equation (1):

$$J = \frac{Q_P}{A} \quad (1)$$

where Q_P is the permeate flow rate (L/h) and A is the surface area of the membrane (m^2). The membrane hydraulic permeability of the membrane is given by Darcy's relation [18]:

$$L_p = \frac{Q_P}{A \times P} \quad (2)$$

where Lp is the membrane hydraulic permeability (L.h^{-1}.m^{-2}.bar^{-1}), Q_P is the permeate flow rate (L/h), A is the surface area of the membrane (m^2) and P is permeate pressure (bar).

The volumetric concentration factor (VCF) was calculated as the ratio of the initial volume of feed divided by the retentate volume at each instant:

$$VCF = \frac{Volume_{initial}}{Volume_{retentate}} \quad (3)$$

2.3. Analytical Methods

2.3.1. Chemical Oxygen Demand (COD) Measurement

COD is the amount of oxygen required by a chemical oxidising agent (typically chromic acid) to oxidise both organic and inorganic substances. COD was measured using the Reactor Digestion Method. An LCK514 COD cuvette test 100–2000 mg/L O_2 (Hach Lange GMBH, Dusseldorf, Germany) was used and the absorbance was measured with a HACH DR3900 Spectrophotometer (Hach Lange GMBH, Dusseldorf, Germany). For some samples, a dilution was required and distilled water was used for that purpose.

2.3.2. Total Protein Content

The Lowry method was used to determine the protein content [19,20]. An aliquot (0.2 mL) of the sample was mixed with 1 mL of an alkaline copper solution and left to react for 10 min. Following the reaction, the mixture was supplied with 0.1 mL of Folin-Ciocalteu reagent. Using a spectrophotometer (Genesys 50, Thermo Scientific, Waltham, MA, USA), the absorbance was measured at 750 nm after 30 min. Protein content in the samples was determined based on a calibration curve using a Bovine serum albumin (BSA) standard at concentrations ranging from 40 to 400 µg/mL. Blank runs were carried out with distilled water instead of the sample.

2.3.3. Total Lipid Content

The total lipids were determined using the Bligh and Dyer method [21]. Succinctly, to 2.5 g of sample, 10 mL methanol and 5 mL chloroform were added. For 2 min, the mixture was forcefully shaken. Next, 5 mL of chloroform was added to the mixture and it was shaken again for 2 min. Distilled water (9 mL) was added and the mixture was vortexed for 2 min. Centrifugation (2000 rpm for 10 min) was used to separate the two phases. The chloroform layer was transferred to a glass vial. A second extraction was carried out with 10 mL of chloroform containing 10% (*v*/*v*) of methanol. The mixture was mixed for 2 min before being centrifuged to separate the two phases. The two chloroform phases were combined and evaporated. The lipid residue was weighed to determine the lipids content of the sample.

2.3.4. SPME/GC-MS

To assess the concentration of aroma compounds in the various condensates collected, gas chromatography analyses were undertaken. A Shimadzu gas chromatograph (GCMS-QP2010, Shimadzu, Japan) equipped with a WAX column (30 m × 0.25 mm i.d. × 0.25 µm) was used during the study. The carrier gas was Ultrapure helium at 1 mL/min. The oven temperature was set at 60 °C (held for 4 min), then 2° C/min to 180° C. The injector temperature was set at 200 °C, which was restricted by the SPME fibre manufacturer's recommended desorption temperature. The detector was adjusted to 220 °C, the ionisation source at 200 °C, and the ionisation mode to electron impact with 70 eV electron energy. A volume of 6 mL was then extracted with a CAR/PDMS fibre at 60 °C for 15 min. The time of analyte desorption from the SPME fibre was fixed at 10 min. For 2 min, the injection was conducted in the splitless mode. After that, until the end of the chromatographic run, the split ratio was set at 1:20. For each parameter understudy, tests were performed in triplicate.

3. Results

3.1. Characterisation of Sardine Cooking Wastewater

The sardine cooking wastewater varies depending on the overall output of the fish canning industry. In order to obtain a representative dataset of wastewater characteristics, several samples (six samplings taken at different times of the year) were collected and analysed. Their characteristics are shown in Table 1.

Table 1. Characterisation of sardine cooking wastewaters.

Parameter	
pH	6.5 ± 0.1
COD (mg/L)	28,080 ± 100
TSS (mg/L)	40,734.64 ± 2520.36
Total protein content (mg/mL)	25.38 ± 1.95
Total lipids content (%)	28.13 ± 2.84

Table 2 presents the aroma profile of the sardine cooking wastewaters, analysed by GC-MS/SPME, comprised mainly of alcohols, aldehydes, and ketones. In minor diversity, it comprises some alkenes, acids, and, in some samples, sulphur compounds. Our results are similar to other studies that analysed the aroma profile of sardine wastewaters [22,23]. These principal classes are predicted as a result of the cooking process, which induces fatty acids to thermally oxidise and decompose, resulting in the formation of several volatile components. Secondary components such as aldehydes, ketones, and alcohols are formed when hydroperoxides decompose due to lipid oxidation [24].

Table 2. Aroma compounds identified in Sardine cooking wastewaters.

Aroma Compounds	Area Ratio (%) *	Concentration (ppm) *
Aldehydes		
Hexanal	9.31%	
Heptanal	2.15%	0.006
2-Hexenal, (E)-	4.36%	
Octanal	1.28%	
Nonanal	5.94%	
2-Octenal, (E)-	2.99%	
2,4-Heptadienal, (E,E)-	6.58%	
2-Nonenal, (E)-	1.63%	0.011
2,6-Nonadienal, (E,Z)-	6.90%	0.044
2-Decenal, (E)-	0.90%	
Alcohols		
1-Penten-3-ol	6.02%	0.100
1-Octen-3-ol	13.69%	0.008
(5Z)-Octa-1,5-dien-3-ol	6.50%	
2-Ethylhexanol	0.57%	
1-Octanol	44.49%	
Sulphur compounds		
Trans-2-(2-Pentenyl)furan	3.52%	
Ketones		
2-Nonanone	3.38%	0.001
3,5-Octadien-2-one	8.47%	
(3E,5E)-3,5-octadien-2-one	8.49%	
2-Undecanone	0.60%	
Acids		
Hexanoic acid	6.71%	

* Mean values of area ratio (%) for all compounds identified and concentration (ppm) of chemical markers. Underlined compounds are off-flavours.

After analysing the aromas present in the different samples, some compounds were identified as chemical markers of our raw material. Chemical markers were chosen based on the major classes of compounds found in wastewaters that have different organoleptic properties. 1-Penten-3-ol was the major compound in all samples obtained and is responsible for the flavour of fresh marine products [22]. 2-penten-1-ol and 1-octen-3-ol were important alcohols in the fresh sardine aroma profile [24,25]. The aldehydes were formed by thermal oxidation and degradation of fatty acids during the cooking step, resulting in the peculiar aroma of cooked fish; these three aromas, in particular, were identified in all samplings [24]. 2-nonanone was the ketone present in all the samplings. These selected markers will be used to compare the different fractions obtained in this study.

Effect of the Antioxidant Extract of Acorn

The effect of acorn extract addition, with antioxidant properties, on the aroma content of sardine cooking wastewater was studied (Figure 4), in order to ensure a minimum deterioration of aromas by oxidation.

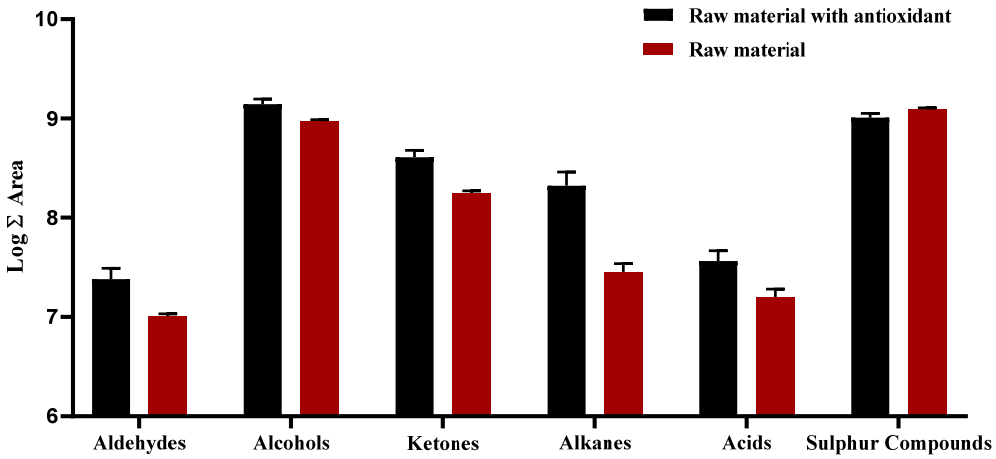

Figure 4. Effect of addition of antioxidant extract on the different classes of aroma compounds identified in sardine condensates.

The results obtained prove that the addition of acorn extract is beneficial, avoiding oxidation of the original aromas, and maintaining the aroma profile of the original samples. The addition of the acorn extract led to a slight reduction in the formation of sulphur compounds, markers of sample degradation, and prevented the oxidation/reduction of aldehydes, which are responsible for the formation of off-flavours. However, this effect is only advantageous when the extract is added at the time of effluent collection. The addition of the extract 24 h after effluent collection, is too late and not effective (data not shown).

3.2. Pre-Treatment: Coagulation/Flocculation Process Pre-Treatment Selection Using Different Combinations of Coagulant and Flocculant

The sardine cooking wastewater was submitted to coagulation/flocculation experiments. The selection of this pre-treatment was motivated by the high investment and energy cost for implementation at full scale of the traditional method, such as centrifugation (analysis not shown).

Control tests using each of the coagulants/flocculants were carried out in order to understand the effectiveness of each one when used independently. Two different combinations of food-grade coagulant/flocculant-chitosan, coupled with carrageenan or alginate, were also tested at different dosages. For these experiments, the pH of the raw wastewater was adjusted to pH 4. At acidic pH, chitosan offers a cationic charge which contributes to colloidal suspension instability and helps to decrease the charge of organic matter, making it less water-soluble [26,27].

- Characterisation of protein and lipid recovery of the fractions obtained with the application of chitosan, carrageenan and alginate used individually

To understand the potential for the clarification of supernatant and the effectiveness of each polysaccharide tested, they were analysed separately and their capacity to recover proteins and lipids was evaluated (Table 3).

Table 3. Protein and lipid recovery obtained by the use of Coagulant or Flocculant applied individually. Analytical data are shown as mean ± SD (n = 3).

Polysaccharide	*Fraction*	*Weight Proportion (%)*	*Protein Recovery in Solid Fraction (%)*	*Lipid Recovery in Solid Fraction (%)*
Chitosan (100 mg/L)	Solid F. Supernatant	4.60 95.40	35.97 ± 2.1	56.02 ± 0.5
Carrageenan (10 mg/L)	Solid F. Supernatant	4.35 95.65	41.77 ± 0.3	54.50 ± 4.0
Alginate (10 mg/L)	Solid F. Supernatant	4.46 95.54	42.32 ± 0.8	40.22 ± 1.5

In the combined coagulation–flocculation process, the coagulation step is described as promoting the instability of the suspension, resulting in aggregation. Chitosan possesses various inherent properties that make it an excellent coagulant, including a high cationic charge density and long polymer chains. Charge neutralisation, adsorption, and precipitative coagulation are some of the mechanisms described for the action of chitosan [15]. Individually, chitosan shows better results in the recovery of lipids than proteins, comparatively with the use of flocculants.

To achieve the settling of large agglomerates from destabilised particles, flocculation is necessary [15] and this is the role of carrageenan and alginate. The results obtained in the control tests show that both flocculants lead to similar percentages of protein recovery and are better than chitosan. However, in terms of lipids, carrageenan showed better results than alginate.

In comparison, carrageenan shows good potential for the clarification of the supernatant, similar to chitosan, requiring a lower concentration. However, several studies report that the use of coagulant/flocculant combinations is significantly more effective [13,28,29].

- Characterisation of protein and lipid content of the fractions, for selection of the best combination of coagulant and flocculant and their concentrations

In the first series of combination experiments, six coagulation–flocculation tests were carried out to evaluate the effect of chitosan/carrageenan concentrations on the clarification of supernatant, resulting in solid fractions with high values of protein and lipid recoveries. To analyse the efficiency of each coagulation/flocculation process to recover nutrients from the sardine cooking wastewater, the recovery of proteins and lipids in the solid fraction was measured and compared to the supernatant fraction (Table 4).

Table 4. Protein and lipid recovery obtained by coagulation/flocculation using different Chitosan/Carrageenan combinations. Analytical data are shown as mean ± SD (n = 3).

Treatment (mg/L)	*Sample*	*Weight Proportion (%)*	*Protein Content (mg/mL)*	*Lipid Content (g/100 g)*	*Protein Recovery in Solid Fraction (%)*	*Lipid Recovery in Solid Fraction (%)*
100/10 *	Solid F. Supernatant	4.66 95.34	30.33 ± 2.84 5.44 ± 0.30	25.26 ± 0.92 10.16 ± 1.73	**79 ± 1**	**64 ± 4**
200/20	Solid F. Supernatant	10.41 89.60	21.19 ± 1.59 5.92 ± 0.55	20.02 ± 2.50 9.08 ± 1.08	77 ± 2	68 ± 3
—300/30	Solid F. Supernatant	6.34 93.66	24.70 ± 0.29 6.80 ± 0.29	26.11 ± 0.44 10.63 ± 3.45	73 ± 1	55 ± 2
400/40	Solid F. Supernatant	5.38 94.62	27.55 ± 1.86 6.47 ± 0.13	18.99 ± 2.26 9.35 ± 2.17	75 ± 1	65 ± 7
500/50	Solid F. Supernatant	10.39 89.61	16.83 ± 1.38 6.87 ± 0.39	7.06 ± 1.21 7.77 ± 1.51	73 ± 2	72 ± 4
600/60	Solid F. Supernatant	8.16 91.84	25.12 ± 2.30 7.28 ± 0.57	16.78 ± 1.44 5.13 ± 0.06	**71 ± 2**	**82 ± 1**

Legend: * 100/10 mg/L means that the concentration of the combination used was 100 mg/L of chitosan and 10 mg/L of carrageenan. The other concentrations are represented in a similar way.

All concentration values tested using this combination showed good results, with a recovery of protein above 70% and lipids above 60%, in the solid fraction. The lowest concentrations tested, 100/10 mg/L, led to the best result for proteins recovery and the highest concentrations tested led to the highest recovery of lipids. Therefore, for the chitosan/carrageenan system, these two combinations were considered the best choice, allowing for high recovery and, simultaneously, a good clarification of the supernatant. These results are in line with the Holland and Shahbaz study [28], where mussel cooking wastewaters were processed. In that study, the use of chitosan alone was compared with a combination of chitosan/carrageenan, leading to a 79% protein recovery with chitosan only and 90% protein recovery with the chitosan/carrageenan combination. Forghani et al. [2] concluded that carrageenan in the lowest concentration (0.45 g/L) tested was the most efficient flocculant in terms of protein sedimentation (≤86% of proteins), in comparison with alginate, when processing shrimp cooking wastewater.

As reported in the literature [13,28,29], the results obtained with the combination of chitosan/carrageenan in the lower concentration range show higher efficiency than the results obtained in the control trials (see Table 3), revealing that the use of the coagulant/flocculant combination is advantageous.

Additionally, six coagulation–flocculation tests were performed to assess the influence of chitosan/alginate concentration on the processing of the original samples. To assess the effectiveness of the coagulation/flocculation method, the protein and lipid recovery were determined (Table 5).

Table 5. Protein and lipid recovery obtained by coagulation/flocculation using different Chitosan/Alginate combinations. Analytical data are shown as mean ± SD (n = 3).

Treatment (mg/L)	*Sample*	*Weight Proportion (%)*	*Protein Content (mg/mL)*	*Lipid Content (g/100 g)*	*Protein Recovery in Solid Fraction (%)*	*Lipid Recovery in Solid Fraction (%)*
***100/10** **	Solid F.	8.56	22.49 ± 1.66	10.98 ± 1.37	**78 ± 3**	**46 ± 2**
	Supernatant	91.44	5.64 ± 0.76	11.37 ± 0.76		
200/20	Solid F.	6.07	19.10 ± 0.27	7.44 ± 0.86	43 ± 6	49 ± 4
	Supernatant	93.93	14.51 ± 1.51	14.40 ± 1.62		
300/30	Solid F.	5.27	34.99 ± 1.66	8.97 ± 3.84	34 ± 4	38 ± 5
	Supernatant	94.73	16.86 ± 0.95	17.52 ± 2.24		
400/40	Solid F.	4.84	31.08 ± 0.59	24.65 ± 0.56	35 ± 2	42 ± 2
	Supernatant	95.16	16.62 ± 0.71	16.25 ± 0.63		
500/50	Solid F.	4.46	**49.37 ± 1.36**	**39.61 ± 1.51**	41 ± 2	34 ± 1
	Supernatant	95.54	15.07 ± 0.51	14.36 ± 0.60		
600/60	Solid F.	5.72	46.78 ± 1.00	24.65 ± 0.11	24 ± 3	44 ± 4
	Supernatant	94.28	19.27 ± 0.91	15.84 ± 3.73		

Legend: * 100/10 mg/L means that the concentration of the combination used was 100 mg/L of chitosan and 10 mg/L of alginate. The other concentrations are represented in a similar way.

For the chitosan/alginate combinations, a good clarification of the supernatant is achieved (confirmed visually). However, compared with the combinations with carrageenan, only for the lowest combination of concentrations (100/10) was it possible to obtain a similar recovery of proteins. Regarding lipids, this combination achieved only a lipid recovery of between 35–49%. Regarding the chitosan/alginate combination, the combinations 100/10 and 500/50 mg/L were selected, due to the higher lipids and protein percentage of recovery, in the first case, and the high protein and lipid content presented in the solid fraction for 500/50 mg/L. Wibowo et al. [29] investigated the effect of chitosan-alginate complexes on surimi wash wastewater recovery and found that at the lowest concentration tested, 100/10 mg/L, higher protein recovery was obtained.

- Characterisation of aroma content in the fractions obtained, for selection of the best combination of coagulant and flocculant

After defining the best concentration for each combination of coagulant/flocculant, all the fractions obtained were analysed by SPME/GC-MS. Figure 5 presents the results for the **chitosan/carrageenan** combination.

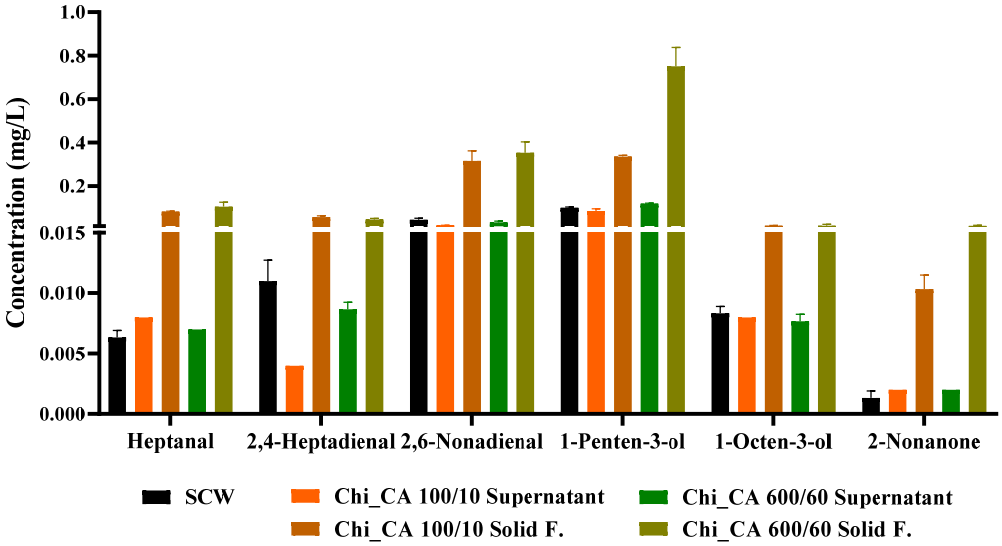

Figure 5. Concentration of the different aromatic markers present in the two fractions obtained by coagulation/flocculation using chitosan/carrageenan. SCW-sardine cooking wastewater.

These results show that the flocculation/coagulation process not only recovers proteins and lipids but aromas present in the original sample as well, probably due to the recognised interactions between aromas and proteins in an aqueous solution [30]. For all samples in both concentration ranges, the aroma content is higher in the solid fraction than in the supernatant, which contributes to the clarification of the supernatant. Liang et al. [31] concluded that chitosan could lead to an effective defatting and deodorisation of oyster hydrolysates, presenting similar results in terms of the aroma content in the raw material and the supernatant after flocculation. For the combination of chitosan/carrageenan the range of concentrations tested does not show differences in the concentrations of aromas that remained in the supernatant.

For the selected concentrations of **chitosan/alginate**, based on the (high) protein and lipid recovery in the solid fraction, the aroma concentration in the supernatant and the solid phase is presented in Figure 6.

As occurred with chitosan/carrageenan combinations, a significant portion of the aroma content was removed from the supernatant along with proteins and lipids. However, the alginate combinations using the lower concentration of coagulant/flocculant show a higher concentration of aromas in the supernatant.

In terms of aroma content, the most concentrated supernatant are the ones obtained with the lowest concentration of chitosan and alginate. However, the solid fraction with a higher concentration of the marker 1-penten-3-ol is the fraction obtained with the highest concentration of coagulant/flocculant.

- Sterilisation of the solid fractions and characterisation of their aroma content

The solid fractions that result from the coagulation/flocculation processes are excellent candidates as ingredients in feed applications, due to their high content of proteins and lipids and their appealing aroma content. Additionally, the presence of chitosan adds biological properties that could be of interest to include in pet food, as referred by Hirano et al. [32] whose study shows an anticholesterol effect of chitosan in rabbits and broilers.

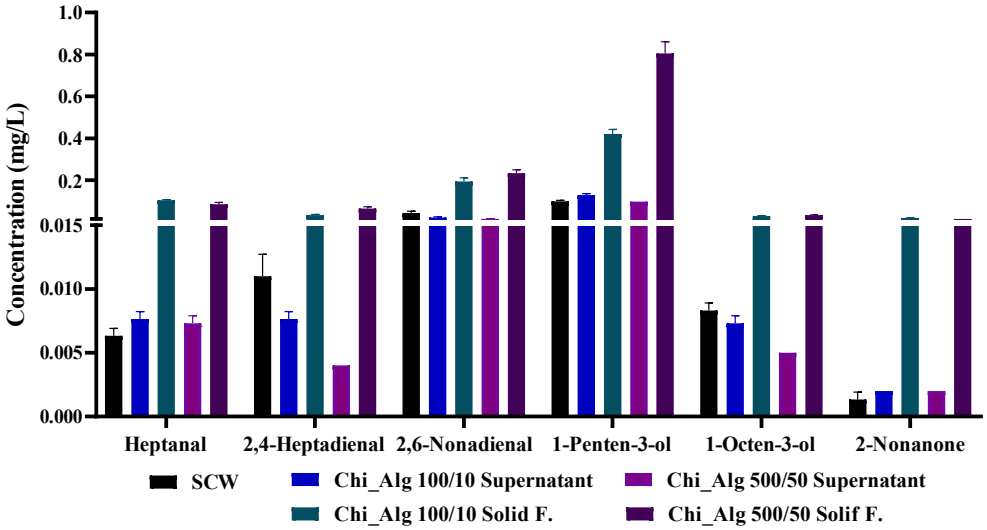

Figure 6. Concentration of the different aromatic markers present in the two fractions obtained by coagulation/flocculation using chitosan/alginate. SCW-sardine cooking wastewaters.

To obtain a safe quality ingredient, the effect of an additional sterilisation step on the texture and aroma content of the solid fractions was evaluated (Figure 7). The choice of a thermal method for sterilising this solid fraction was based on the fact that temperature helps to promote the gelatinisation of the coagulant/flocculant present, improving the final texture of the ingredient. Besides this, it allows for a safe product since a thermal treatment will contribute to reducing the microbial load.

Figure 7. Visual aspect of the selected solid fractions after sterilisation.

After sterilisation, a large informal sensory panel evaluated the sensorial characteristics (such as colour, smell, and texture) of the four solid fractions obtained. A colour change was observed in all samples, which became darker. The fishy smell was present in all combinations, but with different intensities. Some solid fractions present a slightly smoky aroma, as is the case of the highest concentration of chitosan/carrageenan combination. Among the four samples, the one obtained with the highest concentration of chitosan/alginate combination provided the best attractive textural combination among the four samples. Regarding both texture and smell, the chitosan/carrageenan combination at the highest concentration tested proved to be the most suitable choice.

Regarding the effect of the thermal process on the aroma content, the aromas of the chitosan/carrageenan combination at higher concentrations were analysed before and after sterilisation (Figure 8).

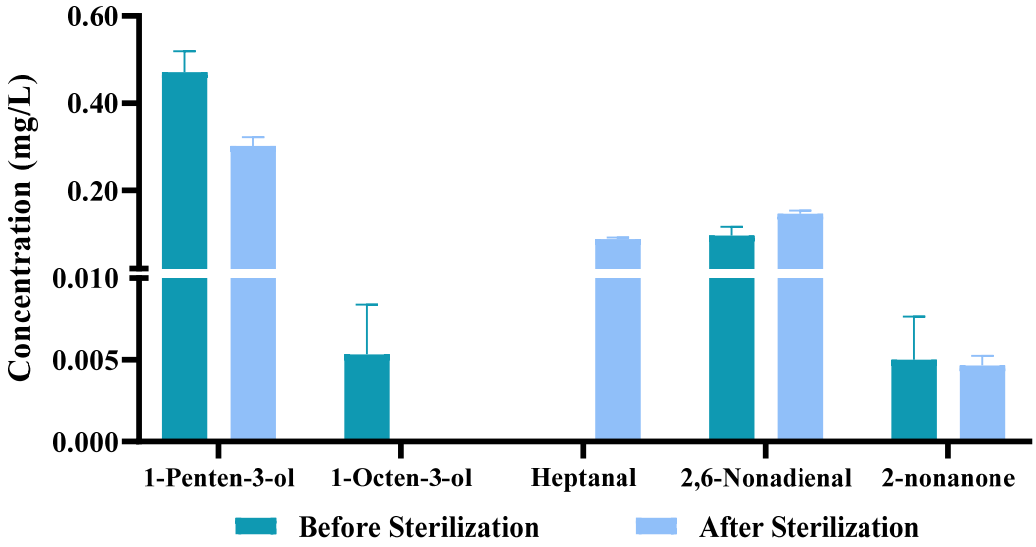

Figure 8. Effect of sterilisation on the concentration of aromas presents in the product obtained with the chitosan/carrageenan combination at higher concentration.

As expected, the thermal method had some negative impact on the aroma content, more extensive in the alcohol and aldehyde families. Due to the temperature effect, some degradation of alcohols was observed, with the loss of 1-octen-3-ol and a decrease in the concentration of the main aroma compound–1-penten-3-ol. The increase in the concentration of heptanal and 2,6-nonadienal may be due to the impact of temperature on lipid oxidation, resulting in an increased release of aldehydes [33]. In future studies, the impact of non-thermal sterilisation methods should be evaluated, for example, by UV treatment. However, the thermal processing shows a positive impact on the texture of the product obtained, which largely compensates for the loss of some aromas.

- Characterisation of the supernatant obtained from the four coagulant/flocculant selected combinations

At the end of the optimisation of the coagulation/flocculation process, these fractions were analysed in order to understand the effect of this pre-treatment on the COD values of the supernatants (Figure 9).

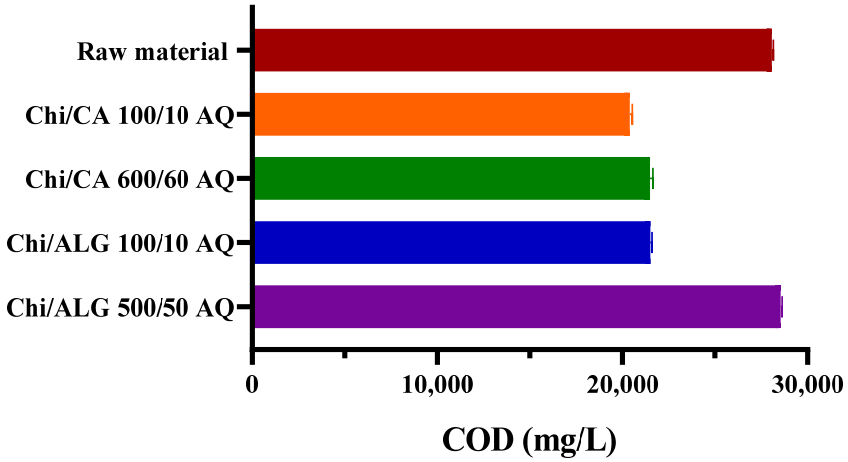

Figure 9. Effect of the coagulation/flocculation process on the COD levels of the supernatants. Note: Legislation allows for effluent discharge for COD values < 2000 mg/L O_2.

The results obtained show that the supernatants still present relatively high levels of natural organic matter. Nevertheless, it can be noticed that there is a decrease in COD of around 25% in relation to the raw material for the chitosan/carrageenan combinations and the lowest concentration for the carrageenan/alginate combination. These results show that even the lowest values of COD obtained are largely above the maximum value of COD allowed for discharge in a collector, for further treatment. It should be noticed that part of this COD is due to the remaining presence of proteins, lipids, and aromas. Therefore, it was decided to process these supernatants by reverse osmosis, which should allow for obtaining a liquid concentrate enriched in organic matter, including aromas, and a permeate that should easily comply with the actual legislation.

3.3. Aroma Recovery by Reverse Osmosis: Selection of the Best Coagulant/Flocculant Combination

A reverse osmosis concentration of the supernatants of two different coagulant/flocculant combinations, at low and high concentrations, was conducted. The initial volume of the feed was 0.6 L. For all combinations tested, a concentration factor of 3 was reached after the reverse osmosis step. Figure 10 shows the permeance data (permeate volume obtained divided by the driving force applied) plotted against the volumetric concentration factor.

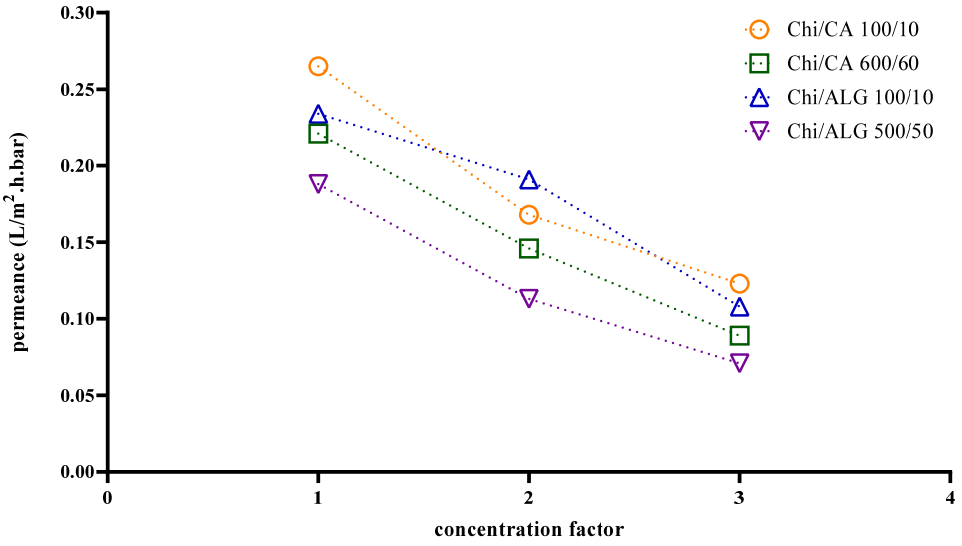

Figure 10. Membrane permeance as a function of volumetric concentration factor for different coagulant/flocculant combinations. Chi—Chitosan; CA—Carrageenan; ALG—Alginate.

This figure shows that with an increase in the concentration factor, the permeance of the membrane reduces progressively. This is expected behaviour due to the increase in the concentration of foulant compounds present in the solution [34,35]. The highest permeance was recorded for the chitosan/carrageenan combination at the lowest concentration (0.123–0.265 L/m^2 h), and the lowest for the chitosan/alginate combination with the highest concentration (0.071–0.188 L/m^2 h). For chitosan/carrageenan, the processing time required to achieve a volumetric concentration factor of 3 was 1 h shorter when using the lowest concentration of coagulant/flocculant. The processing time required increases significantly (4 h more) with the increase in concentration when using the chitosan/alginate combination. The significant permeance decrease in the high range of alginate might be explained by the fact that alginate, due to the preferential binding between calcium and carboxylate groups of alginates, may form a gel layer network on the membrane surface in the presence of calcium ions [36].

The protein and lipid content in all combinations were also studied (Figure 11). The protein amount was higher in retentate, which was expected due to the retention of proteins by the reverse osmosis membrane. However, the protein concentration factor achieved in the retentates did not match the volumetric concentration factor of 3, which suggests that part of the proteins are adsorbed to the membrane surface, contributing to the fouling effect observed.

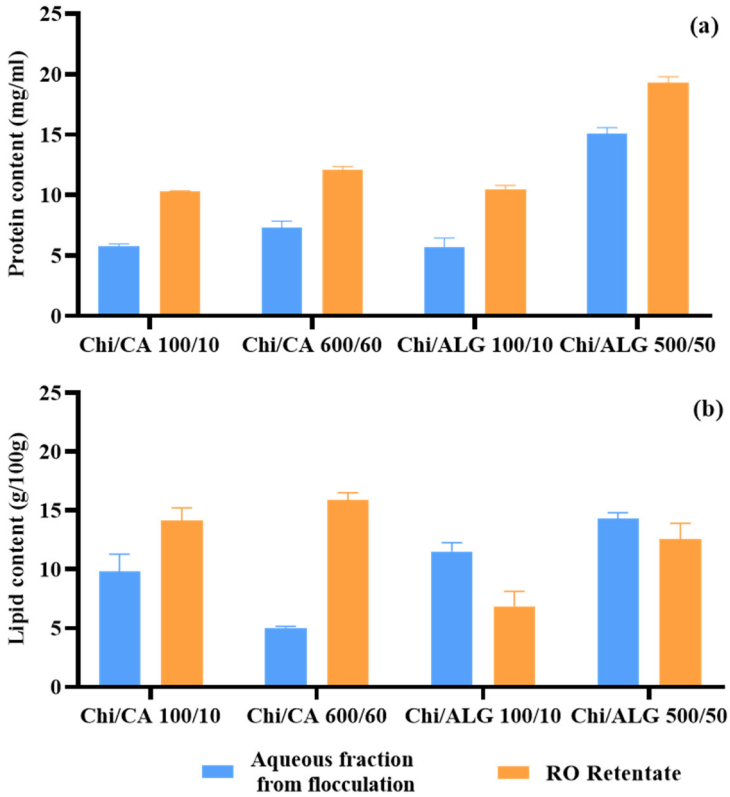

Figure 11. Protein (**a**) and lipid (**b**) content of reverse osmosis retentates, for different combinations of chitosan/carrageenan and chitosan/ alginate.

The results obtained for the retention of lipids by reverse osmosis are more complex to interpret. Except for the results obtained for Chitosan/Carrageenan, the results obtained suggest high adsorption of lipids at the membrane surface. Actually, for the Chitosan/Alginate combination, alginate might be bound to lipids, forming aggregates that may adsorb to the membrane, forming a film.

The aromas recovered in the retentates of different coagulant/flocculant combinations were analysed (Figure 12). 1-Penten-3-ol is still the main compound present in all combinations.

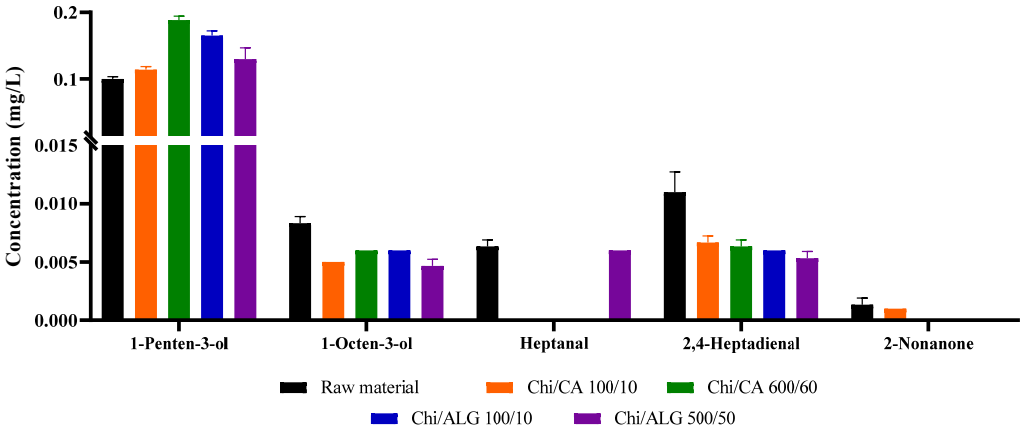

Figure 12. Aroma characterisation of the RO retentates for different coagulation/flocculation combinations.

Focusing on the obtained permeates, Figure 13 presents the values of COD obtained, in order to evaluate the possibility of reusing the permeate as process water.

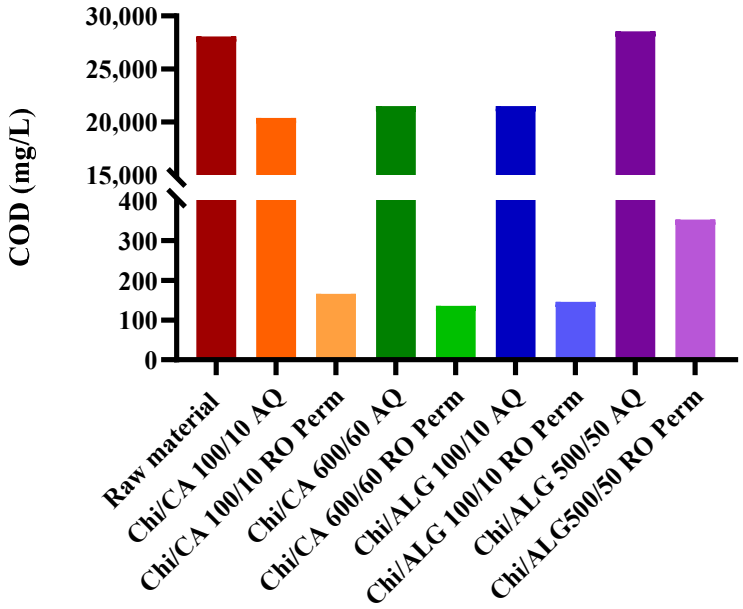

Figure 13. Effect of integrated system of coagulation/flocculation followed by reverse osmosis on the COD of the permeates.

It should be stressed that, in terms of COD content, all permeates obtained have rather low COD levels, below 400 mg/L, which allows for the direct discharge of this stream to a public wastewater collector system. Additionally, these permeates, due to the fact that they do not present protein or lipid content and only a residual presence of aromas (representing only 2% of the total area of aromas in the sample), can be considered for reuse at the production site, namely for washing operations, leading to significant savings in freshwater consumption.

At the end of the optimisation of the coagulation/flocculation process, we found that the best combination was chitosan/carrageenan at the highest concentration tested (60/600 mg/L). This combination leads to the highest recovery of lipid content from the initial sample and a reduction of protein content above 70% in the coagulation/flocculation process. It allows for producing a solid fraction with a high protein content (25.12 mg/mL) and the best texture obtained. Also, the average permeance in the reverse osmosis process for the chitosan/carrageenan at the highest concentration tested (60/600 mg/L) is higher (0.169 L/m^2.h.bar) than for the chitosan/alginate process in the high concentration range.

Additionally, this combination allows for and leads to the permeate with the lowest COD load (136 mg/L O_2), allowing the reuse of water in other stages of the process.

4. Conclusions

The integrated Coagulation–Flocculation/Reverse Osmosis process proposed in this paper has proven to be an efficient method for the valorisation of sardine cooking waters produced by the canning industry. The implementation of this process allows for the obtaining of two different products for use as ingredients in feed for pets and also for aquaculture: a solid fraction rich in proteins and lipids (above 20% *w*/*w*) and a liquid aroma concentrate, which can be applied as a natural flavouring for food or feed products. Additionally, it avoids the generation of effluent with a high organic load and makes possible the recovery of water for reuse in the production process.

The combination of chitosan as coagulant at a concentration of 600 mg/L and carrageenan as flocculant at a concentration of 60 mg/L was demonstrated to be the most efficient for proteins and lipids recovery and, after the reverse osmosis step, it also revealed better permeance to obtain water with quality to be reused in other steps of canning processing.

The process proposed, allows for the production of two added-value products—a solid fraction rich in proteins and lipids and a condensate rich in aroma compounds—combined with the reuse of resulting clean(er) water.

Author Contributions: Conceptualisation, M.J.P. and C.B.; methodology, M.J.P., M.P., and C.B.; investigation, M.J.P. and O.G.; resources, J.C.; writing—original draft preparation, M.J.P.; writing—review and editing, M.P., J.C., and C.B.; supervision, M.P., J.C., and C.B.; project administration, J.C. and C.B.; funding acquisition, J.C. and C.B. All authors have read and agreed to the published version of the manuscript.

Funding: The research leading to these results has received funding from Sistema de Apoio à Investigação Científica e Tecnológica (SAICT), Programa de Actividades Conjuntas (PAC) under Multibiorefinery Project (POCI-01-0145-FEDER-016403) and from European Regional Development Fund (ERDF), through the Incentive System to Research and Technological Development, within the Portugal2020 Competitiveness and Internationalization Operational Program under the project "MobFood–Mobilizing scientific and technological knowledge in response to the challenges of the agri-food market" (POCI-01-0247-FEDER-024524). Maria João Pereira acknowledges Fundação para a Ciência e Tecnologia for PhD fellowship SFRH/BD/119018/2016. This work was supported by the Associate Laboratory for Green Chemistry–LAQV, which is financed by national funds from FCT/MCTES (UIDB/50006/2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Omil, F.; García-Sandá, E.; Méndez, R.; Lema, J.M. Clean technologies for wastewater management in seafood canning industries. In *Technological Choices for Sustainability*; Springer: Berlin/Heidelberg, Germany, 2014; pp. 103–125. [CrossRef]
2. Forghani, B.; Sørensen, A.D.M.; Johannesson, J.; Svendsen, T.C.; Undeland, I. Flocculation and Flotation to Recover Protein-Enriched Biomasses from Shrimp Boiling and Peeling Process Waters: A Case Study. *ACS Sustain. Chem. Eng.* **2020**, *8*, 9660–9668. [CrossRef]
3. INE (Instituto National de Estatística). *Estatísticas da Pesca 2011*; Instituto Nacional de Estatística: Lisbon, Portugal, 2012; ISBN 9789892501567.
4. Almeida, C.; Vaz, S.; Ziegler, F. Environmental Life Cycle Assessment of a Canned Sardine Product from Portugal. *J. Ind. Ecol.* **2015**, *19*, 607–617. [CrossRef]
5. Cristóvão, R.; Botelho, C.; Martins, R.; Boaventura, R. Pollution prevention and wastewater treatment in fish canning industries of Northern Portugal. *Int. Proc. Chem. Biol. Environ. Eng.* **2012**, *32*, 12–16. [CrossRef]

6. Venugopal, V.; Sasidharan, A. Seafood industry effluents: Environmental hazards, treatment and resource recovery. *J. Environ. Chem. Eng.* **2021**, *9*, 104758. [CrossRef]
7. Cristóvão, R.O.; Botelho, C.M.; Martins, R.J.E.; Loureiro, J.M.; Boaventura, R.A.R. Fish canning industry wastewater treatment for water reuse—A case Study. *J. Clean. Prod.* **2015**, *87*, 603–612. [CrossRef]
8. Hung, Y.-T.; Show, K.-Y.; Tay, J.-H. Seafood Processing Wastewater Treatment. In *Handbook of Industrial and Hazardous Wastes Treatment*; CRC Press: Boca Raton, FL, USA, 2005; pp. 706–749. [CrossRef]
9. Galanakis, C.M. Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends Food Sci. Technol.* **2012**, *26*, 68–87. [CrossRef]
10. Bourseau, P.; Massé, A.; Cros, S.; Vandanjon, L.; Jaouen, P. Recovery of aroma compounds from seafood cooking juices by membrane processes. *J. Food Eng.* **2014**, *128*, 157–166. [CrossRef]
11. Vandanjon, L.; Cros, S.; Jaouen, P.; Quéméneur, F.; Bourseau, P. Recovery by nanofiltration and reverse osmosis of marine flavours from seafood cooking waters. *Desalination* **2002**, *144*, 379–385. [CrossRef]
12. Cai, Y.H.; Galili, N.; Gelman, Y.; Herzberg, M.; Gilron, J.; Mohseni, A.; Kube, M.; Fan, L.; Roddick, F.A. Treatment of wastewater reverse osmosis concentrate using alginate-immobilised microalgae: Integrated impact of solution conditions on algal bead performance. *J. Memb. Sci.* **2021**, *623*, 119054. [CrossRef]
13. Arias-Lizárraga, D.M.; Méndez-Gomez, E. Remoción de sólidos en aguas residualesde la industria harinera de pescado empleando biopolímeros. *Tecnol. Ciencias Del Agua* **2014**, *5*, 115–123.
14. Venugopal, V. Valorization of Seafood Processing Discards: Bioconversion and Bio-Refinery Approaches. *Front. Sustain. Food Syst.* **2021**, *5*, 611835. [CrossRef]
15. Teh, C.Y.; Budiman, P.M.; Shak, K.P.Y.; Wu, T.Y. Recent Advancement of Coagulation-Flocculation and Its Application in Wastewater Treatment. *Ind. Eng. Chem. Res.* **2016**, *55*, 4363–4389. [CrossRef]
16. Tremblay, A.; Corcuff, R.; Goulet, C.; Godefroy, S.B.; Doyen, A.; Beaulieu, L. Valorization of American lobster (Homarus americanus) cooking waters: Preparation and characterization of a food ingredient. *J. Food Process. Preserv.* **2021**, *45*, e15665. [CrossRef]
17. Tremblay, A.; Corcuff, R.; Goulet, C.; Godefroy, S.B.; Doyen, A.; Beaulieu, L. Valorization of snow crab (Chionoecetes opilio) cooking effluents for food applications. *J. Sci. Food Agric.* **2020**, *100*, 384–393. [CrossRef] [PubMed]
18. Mulder, M.; Mulder, J. *Basic Principles of Membrane Technology*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; Volume 148, ISBN 079234247X.
19. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275. [CrossRef]
20. Barbarino, E.; Lourenço, S.O. An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. *J. Appl. Phycol.* **2005**, *17*, 447–460. [CrossRef]
21. Manirakiza, P.; Covaci, A.; Schepens, P. Comparative Study on Total Lipid Determination using Soxhlet, Roese-Gottlieb, Bligh & Dyer, and Modified Bligh & Dyer Extraction Methods. *J. Food Compos. Anal.* **2001**, *14*, 93–100. [CrossRef]
22. Ganeko, N.; Shoda, M.; Hirohara, I.; Bhadra, A.; Ishida, T.; Matsuda, H.; Takamura, H.; Matoba, T. Analysis of volatile flavor compounds of sardine (Sardinops melanostica) by solid phase microextraction. *J. Food Sci.* **2008**, *73*, S83–S88. [CrossRef]
23. Mansur, M.A.; Bhadra, A.; Takamura, H.; Matoba, T. Volatile flavor compounds of some sea fish and prawn species. *Fish. Sci.* **2003**, *69*, 864–866. [CrossRef]
24. Chung, H.; Choi, A.; Cho, I.H.; Kim, Y.; Korea, S. Changes in fatty acids and volatile components in mackerel by broiling. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 1481–1490. [CrossRef]
25. Martínez, R.; Sanz, M.T.; Beltrán, S. Concentration by pervaporation of representative brown crab volatile compounds from dilute model solutions. *J. Food Eng.* **2011**, *105*, 98–104. [CrossRef]
26. Nimesha, S.; Hewawasam, C.; Jayasanka, D.J.; Murakami, Y.; Araki, N.; Maharjan, N. Effectiveness of natural coagulants in water and wastewater treatment. *Glob. J. Environ. Sci. Manag.* **2022**, *8*, 101–116. [CrossRef]
27. Jouenne, E.; Crouzet, J. Effect of pH on retention of aroma compounds by β-lactoglobulin. *J. Agric. Food Chem.* **2000**, *48*, 1273–1277. [CrossRef] [PubMed]
28. Holland, C.R.; Shahbaz, M. Mussel protein recovery using dissolved air flotation. *J. Food Eng.* **1986**, *5*, 135–151. [CrossRef]
29. Wibowo, S.; Velazquez, G.; Savant, V.; Torres, J.A. Effect of chitosan type on protein and water recovery efficiency from surimi wash water treated with chitosan-alginate complexes. *Bioresour. Technol.* **2007**, *98*, 539–545. [CrossRef]
30. Rogacheva, S.; Espinosa-Diaz, M.A.; Voilley, A. Transfer of aroma compounds in water-lipid systems: Binding tendency of β-lactoglobulin. *J. Agric. Food Chem.* **1999**, *47*, 259–263. [CrossRef]
31. Liang, S.; Zhang, T.; Fu, X.; Zhu, C.; Mou, H. Partially degraded chitosan-based flocculation to achieve effective deodorization of oyster (Crassostrea gigas) hydrolysates. *Carbohydr. Polym.* **2020**, *234*, 115948. [CrossRef]
32. Hirano, S.; Itakura, C.; Seino, H.; Akiyama, Y.; Nonaka, I.; Kanbara, N.; Kawakami, T. Chitosan as an Ingredient for Domestic Animal Feeds. *J. Agric. Food Chem.* **1990**, *38*, 1214–1217. [CrossRef]
33. Peinado, I.; Miles, W.; Koutsidis, G. Odour characteristics of seafood flavour formulations produced with fish by-products incorporating EPA, DHA and fish oil. *Food Chem.* **2016**, *212*, 612–619. [CrossRef]
34. Destani, F.; Naccarato, A.; Tagarelli, A.; Cassano, A. Recovery of aromatics from orange juice evaporator condensate streams by reverse osmosis. *Membranes* **2020**, *10*, 92. [CrossRef]

35. Pozderović, A.; Popović, K.; Pichler, A.; Jakobek, L. Influencia de los parámetros de procesamiento en el flujo de permeación y la retención de compuestos aromáticos y fenólicos en el zumo concentrado de arándano silvestre mediante ósmosis inversa. *CYTA-J. Food* **2016**, *14*, 382–390. [CrossRef]
36. Xie, Z.; Nagaraja, N.; Skillman, L.; Li, D.; Ho, G. Comparison of polysaccharide fouling in forward osmosis and reverse osmosis separations. *Desalination* **2017**, *402*, 174–184. [CrossRef]