NOVA SCHOOL OF SCIENCE & TECHNOLOGY

DEPARTAMENT OF CHEMISTRY

RECOVERY OF NATURAL FLAVOURS BY INTEGRATED MEMBRANE PROCESSING AND FRACTIONATED CONDENSATION

CASE STUDY OF SEAFOOD AND WINE INDUSTRIES

MARIA JOÃO LOUREIRO DO VALLE PEREIRA

Master's in Biotechnology

DOCTORATE IN SUSTAINABLE CHEMISTRY
NOVA University Lisbon
SEPTEMBER 2022

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DOCTORATE IN SUSTAINABLE CHEMISTRY
NOVA University Lisbon
September 2022

Recovery of natural flavours by integrated membrane processing and fractionated condensation: case study of seafood and wine industries

Para a Luísa

ACKNOWLEDGEMENTS

A realização desta tese contou com importantes incentivos e contributos, sem os quais não se teria tornado possível concluir mais este ciclo.

Ao terminar esta importante jornada gostaria de agradecer, em primeiro lugar, à minha orientadora, a Doutora Carla Brazinha, por todo o apoio desde o momento da candidatura até à escrita desta tese. Por me ajudar a compreender todo um novo mundo que tantas vezes me desafiou ao longo destes anos. Obrigada por todo o tempo, interesse e pelo incentivo de fazer mais e melhor.

Ao Professor João Paulo Crespo, pela simpatia e disponibilidade com que me recebeu desde o início, e pela compreensão nas diferentes fases do processo. E pelas valiosas sugestões e discussões científicas que nos fazem questionar e evoluir o nosso trabalho.

À Professora Manuela Pintado, por todo o carinho em todo o meu trajeto na investigação. Foi com quem dei os meus primeiros passos na investigação e dos quais tenho muito a agradecer, das oportunidades a todos os ensinamentos e formas de ver a ciência. Nesta fase específica, pelo apoio nos projetos MobFood e MultiBioRefinery e pelo olhar externo muito importante para a evolução do trabalho.

À Fundação para a Ciência e a Tecnologia pela bolsa de doutoramento atribuída, que permitiu a realização deste trabalho.

Obrigada a todo o grupo de membranas, em especial à Carla Martins, à Paloma, à Inês Meireles, à Sofia, ao Jorge e à Mafalda Cadima. Obrigada pelo apoio dado à mínima dificuldade, por tornarem o período passado no laboratório divertido, por se terem tornarem bons amigos e apoiarem em tempos atribulados. Em especial à Joana Monte e Rita Valério, por estarem sempre lá! Por serem as amigas que melhor perceberam as minhas frustrações e festejaram cada vitória ao longo deste doutoramento, mesmo já estando noutras fases da vida. Por todas as conversas, das descontraídas às científicas, pelas risotas para aliviar o stress e por toda a ajuda na minha descoberta deste mundo da engenharia.

Obrigada a todo o grupo dos Nutracêuticos, antiga família, por todos os momentos partilhados na Plataforma e por toda a disponibilidade e ajuda. Em especial ao Agostinho, Miguel e Ana Nunes pela disponibilidade e apoio na realização do trabalho, assim como um ombro amigo! À Ana Nunes e Ana Roda, por todos os momentos partilhados dentro e fora do laboratório, tornaram-se amigas que vou levar para a vida.

Obrigada aos meus amigos de sempre que mesmo à distância se mantiveram perto, em especial à Ana, à Jessica, à Rita e Rute.

Muito obrigada à minha família, por nunca me ter cobrado as ausências nas datas mais importantes. Aos meus pais que sempre me apoiaram nas minhas escolhas, pelas palavras de conforto e encorajamento que me deram ânimo para continuar e por acreditarem em mim incondicionalmente. À minha irmã que esteve sempre lá para me ouvir e para dar uma palavra amiga e de confiança.

Por último, mas os que mais sofreram comigo ou com as minhas ausências. Obrigada ao Pedro, por ter sido o meu porto de abrigo e pelo companheirismo em todas as fases. Por toda a paciência nos meus dias difíceis e facilitares o dia-a-dia. E claro, à Luísa que literalmente cresceu juntamente com este doutoramento e que com as suas conquistas, abraços e sorrisos me deu forças para concluir esta etapa.

Muito Obrigada!

"And once the storm is over, you won't remember how you made it through, how you managed to survive. You won't even be sure, whether the storm is really over. But one thing is certain. When you come out of the storm, you won't be the same person who walked in. That's what this storm's all about."

(Haruki Murakami, Kafka on the Shore)

ABSTRACT

The work presented in this PhD thesis aims at designing integrated systems to capture natural aromas comprising the recovery and fractionation of natural aromas, as natural flavourings to be added to food products.

Within the seafood case study, canning industry effluents have a high organic matter load, with proteins, aromas and oils, resulting in an environmental impact and economic loss if they are recovered. An integrated process of coagulation/flocculation followed by reverse osmosis was designed which allows for the recovery of protein, lipid and aromas, to reduce the environmental impact of these effluents and reduce the water consumption of this industry, besides the production of aroma natural flavourings.

The recovery of natural flavours, assuring the removal of off-flavours, by integrated membrane processing and fractionated condensation was addressed for two different case studies – i) Sardine cooking wastewaters/seafood model solution and ii) red wine model fermentation headspaces.

An integrated process of pervaporation and fractionated condensation is proposed for the aroma recovery and fractionation from a designed model solution mimicking the aqueous effluents of the seafood industry, combining the advantages of organophilic pervaporation and fractionated condensation. A mathematical model was developed to predict the separation efficiency of the fractioned condensation processing step.

Due to the lack of studies addressing the influence of real food matrices on integrated organophilic pervaporation/fractionated condensation processes, the mathematical model was applied to this integrated system to analyse the impact of the real matrix of sardine cooking wastewaters on the fractionation of aromas.

For red wine fermentation headspaces, the applicability of the mathematical model is assessed for two different alternative processes: fractionated condensation and vapour permeation–fractionated condensation. The aromas of the headspace of red wine fermentation are

commonly lost, through the fermenter venting system, which is enhanced by the stripping effect of the produced CO_2. Both processes studied allow for a good recovery of esters. However, the integrated process of vapour permeation-fractionated condensation achieves a significant decrease in the amount of ethyl phenols.

The major contribution of this PhD thesis is the mathematical model applied and validated that could predict fractionated condensation with only a minimum experimental work and the use of trustworthy thermodynamic parameters. The model is applicable to diverse effluents, including those with components that constrain the condensation process, namely non-condensable gases, and has been verified for both model and real solutions. This model represents a tool that can be readily adapted to other streams from diverse industrial processes for the recovery aromas, following a circular economy approach.

Keywords: aroma recovery, pervaporation/vapour permeation, fractionated condensation, valorisation of food waste, mathematical modelling

RESUMO

O trabalho desenvolvido nesta tese de doutoramento visa projetar sistemas integrados de recuperação de aromas, incluindo a recuperação e fracionamento de aromas naturais, como agentes aromatizantes naturais para serem incorporados em produtos alimentares.

No âmbito do estudo de caso dos aromas de peixe e marisco, os efluentes da indústria conserveira têm uma elevada carga em matéria orgânica, contendo proteínas, lípidos e aromas, resultando num elevado impacto ambiental e numa perda económica, se não forem recuperados. Neste projeto de doutoramento foi concebido um processo integrado de coagulação/floculação seguido de osmose inversa que permite a recuperação de proteínas, lípidos e aromas, reduzindo o impacto ambiental destes compostos e o consumo de água, assegurando a produção de aromas naturais com valor comercial.

A recuperação de aromas naturais com remoção de aromas indesejáveis através do processamento integrado de membranas e condensação fracionada foi abordada para dois casos de estudo diferentes - i) solução modelo de águas residuais da cozedura de sardinha/ solução modelo de aromas de peixe e marisco e ii) solução modelo de aromas libertados na fermentação de vinho tinto.

Foi proposto um processo integrado de pervaporação e condensação fracionada para a recuperação de aromas e seu fracionamento a partir de uma solução modelo concebida de forma a mimetizar os efluentes aquosos da indústria conserveira, combinando as vantagens da pervaporação organofílica e da condensação fracionada. Foi igualmente desenvolvido um modelo matemático para prever a eficiência de separação da etapa de processamento da condensação fracionada.

Devido à falta de estudos sobre a influência de matrizes alimentares reais nos processos integrados de pervaporação organofílica/condensação fracionada, o modelo matemático foi aplicado a este sistema integrado com o objetivo de analisar o impacto da matriz real do efluente de cozedura de sardinha no fracionamento de aromas.

Para o caso de estudo dos aromas libertados na fermentação de vinho tinto, a aplicabilidade do modelo foi avaliada para dois processos alternativos diferentes: condensação fracionada e sistema integrado de permeação de vapor seguido de condensação fracionada. Os aromas libertados na fermentação do vinho tinto são comumente perdidos através do sistema de ventilação do fermentador e são arrastados pelo CO_2 produzido. Ambos os processos estudados permitem uma boa recuperação dos ésteres. No entanto, o processo integrado de condensação fracionada por permeação de vapor conduz a uma diminuição significativa da quantidade de etil fenóis.

A principal contribuição desta tese de doutoramento foi o teste e validação do modelo matemático desenvolvido para previsão do processo de condensação fracionada, usando um mínimo de dados experimentais e com recurso a parâmetros termodinâmicos confiáveis. O modelo é aplicável a diversos efluentes, incluindo aqueles com componentes que restringem o processo de condensação, como é o caso de gases não condensáveis, e foi validado tanto para soluções modelo como para soluções reais. Este modelo representa uma ferramenta que pode ser facilmente adaptada a outros efluentes de outros processos industriais, numa perspetiva de economia circular

Palavras-chave: Recuperação de aromas, pervaporação/permeação de vapor, condensação fracionada, valorização de resíduos alimentares, modelação matemática

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LIST OF ACRONYMS

Abbreviations

4-EG 4-Ethyl Guaiacol

4-EP 4-Ethyl phenol

ALG Alginate

CA Carrageenan

Chi Chitosan

COD Chemical oxygen demand

ED Electrodialysis

MF Microfiltration

MW Molecular weight

NF Nanofiltration

OAV Odour activity value

PDMS Polydimethylsiloxane

PV Pervaporation

RO Reverse osmosis

SPME/GC-MS Solid-phase microextraction followed by gas chromatography-mass spectrometry method

UF Ultrafiltration

VCF Volumetric concentration factor

VP Vapour permeation

Variables

%Condens percentage of condensation [%]

0 between the pervaporation module and the first condenser

1 in the first condenser

1' between condensers

A – membrane area (m^2)

A first constant of the Antoine law [-]

B second constant of the Antoine law [K or °C]

C third constant of the Antoine law [K or °C]

D_i Diffusion coefficient [m^2/s]

feed in the feed side

H_i Henry's law constant of compound i [-]

i compound *i*

inert inert gases

J permeate flux [g m^{-2} h^{-1}] or [mol m^{-2} h^{-1}]

j compound *j*

$k_{ov,i}$ Overall mass transfer coefficient [mol.m^{-2}. s^{-1}. Pa^{-1}]

L_i permeability [mol m^{-1} s^{-1} Pa^{-1}]

L_p permeance [L.h^{-1}.m^{-2}.bar^{-1}]

n molar flow rate [mol s^{-1}]

perm in the permeate side

p_i partial pressure of compound *i* [Pa]

p_{perm} total permeate pressure [Pa] or [mbar]

p_{vi} saturation vapour pressure of pure compound *i* [Pa]

Re_{feed} Reynolds number in the feed compartment [-]

Si Sorption coefficient [-]

T temperature [K] or [◦ C]

t time [s]

V volume [mL]

w water

x liquid phase molar fraction [-]

y gas phase molar fraction [-]

Greek symbols

α selectivity [-]

δ thickness of membrane [m]

Υ^{∞}_{i} activity coefficient of compound i in infinite dilute aqueous solution [-]

1.
BACKGROUND AND MOTIVATION

1.1. Background and Motivation

Food and beverage effluents are residues with a high organic content generated during the processing of raw materials and may be solid, liquid or vapour [1]. However, the possibility of valorising these effluents might contribute to the increase in profitability of the food and beverages industries if adequate processes are established and validated. Since these residues may be regarded as the source of valuable compounds, resulting in new products with a commercial value, the term "by-products" and "co-products" is increasingly employed [2], in a circular economy approach.

Food and beverage industries produce liquid/vapour effluents rich in volatile organic compounds and their direct discharge is unwanted, as it contributes to environmental pollution [3]. Due to fermentation and cooking/thermal processes, they are the generation and transformation of aroma compounds occur, which impact on the organoleptic properties of the final product [4]. To comply with the evolution of environmental regulations [5] and to guarantee the quality of the final product for consumers [6], food industries are focusing on the recovery of the aromas from effluents before their discharge.

The aroma is one of the most important features in the consumer sensory experience and it is directly linked to the quality of the product. Their recovery would also require their valorisation by enhancing the organoleptic properties of the finished goods or their use in other products, as well as reducing the environmental impact of their direct discharge [3], [7]. The market for natural aromas is anticipated to increase in value by 5.7% from 2018 to 2026, reaching US$ 11,269.7 billion. Natural aromas are usually derived from ingredients such as meat, seafood, eggs, dairy products, poultry, vegetable, fruits, bark, bud, root, beverages and other organic sources through distillation or fermentation. A few of the important trends in the worldwide market for natural flavours include rising consumer health consciousness and a focus on flavour distinctiveness with patent protection in processed foods and drinks [8].

Aroma recovery is not an easy task because they are usually highly diluted in complex matrices, formed by a mixture of hundreds of different organic compounds, present at very

low concentrations. Moreover, aroma fractionation may be needed when aiming at recovering a particular aroma or a group of aroma compounds [9], [10].

One of the challenges of the valorisation of aromas from agro-industrial effluents is the presence of off-flavours in these media. An off-flavour is an unpleasant aroma that deteriorates the quality of a food product, even though is often safe, leading to brand damage and adverse publicity, which may be extremely costly to the food or beverage industries [11]. Food and beverage off-flavours can result from: i) chemical reactions from internal sources (e.g., lipid oxidation, enzymatic action) and ii) contamination from external sources, such as microbial metabolic reactions [12]. Thermal processing is responsible for triggering the Maillard reaction or lipid oxidation, which generates many food aromas and some off-flavours. For this reason, the demand for non-thermal treatments or applying milder conditions is emerging [11]. It is worth noting the difficulty of fractioning off-flavours from desirable aromas and the lack of studies on the reduction/removal of these compounds.

Distillation, evaporation, and freeze-crystallization are three of the most used industrial separation techniques for liquid separation in the food industry, but they are disadvantageous high energy-intensive processes. As a result, improvements to the present separation methods are required [13], [14]. Membrane separation technologies have been developed as separation and purification processes applied in a variety of fields, including chemical, petrochemical, biochemical, pharmaceutical, environmental, food, and beverage [15].

Two of the most important food industries in the Portuguese economy are the fish-canning and wine industries. For both industries, it would be relevant from an environment and an economic point of view to develop processes for the recovery of natural aromas assuring off-flavours removal, overcoming the challenge of processing effluents complex feedstocks with a high diversity of aromas pertaining to different chemical families. Effluents from seafood processing have the additional complexity of having a high content of lipids, proteins and off-flavours resulting from the cooking processing step. The red wine effluent (headspace) has the additional complexity of comprising ethanol, CO_2 and off-flavours resulting from microbial contamination processes.

1.1.1. Membrane processing

Membrane separation is a valuable approach for processing concentrate aroma distillates and natural extracts due to its advantages over evaporation and other traditional concentration procedures in terms of mild conditions of temperature, low energy intensity, and without involving the addition of any mass agents such as solvents. Membrane processes are an effective approach for minimising heat damage of aroma compounds, which are heat-sensitive molecules, and avoiding product contamination while preserving the biological activity of the compounds recovered [16], [17].

The design of integrated processes, that link multiple membrane processes or combine membrane operations with traditional separation technologies, provides synergies that cannot be obtained when establishing as a single process [18], [19]. Three different membrane processes have been studied during this PhD project for aroma recovery and concentration/fractionation, specifically reverse osmosis (RO), pervaporation (PV) and vapour permeation (VP).

Reverse osmosis is a liquid-phase process in which the applied transmembrane pressure causes preferential movement of solvent. The use of dense membranes enables the concentration of aromas due to the good retention of small solutes, including most ions and low molecular mass compounds present in water. The membrane's high hydrophilicity allows water to efficiently permeate through the polymer structure of the membrane when a differential hydrostatic pressure is established between the two sides of the membrane [2], [20], [21]. In addition to its status as a leading technology for seawater desalination, RO has been highlighted as a technology of substantial interest for various applications such as wastewater treatment, and food and beverage processing. Due to the possibility of working at low temperatures, it offers several advantages over previous technologies in the food and beverage industry, including the preservation of thermally sensitive components, lower investment costs, lower energy use, and the retention of aroma compounds [22].

Vacuum pervaporation is a membrane technique where a dense membrane separates a liquid feed solution from a vapour permeate, where vacuum conditions are applied to the permeate compartment. The transport across the membrane barrier is based on compound-membrane interactions [23], [24]. The mass transfer occurs in three steps, according to the solution-diffusion model: i) sorption of the permeant from the feed liquid to the membrane; ii) diffusion of the permeant across the membrane; and iii) desorption of the permeant from the membrane to the vapour phase at the permeate side [25], [26]. The permeants are separated because of their differences in solubility in the material of the membrane top-layer and the differences in the rates at which different permeants diffuse through the membrane [27]–[29]. In the majority of cases (although not always) the desorption step is negligible in terms of mass transport of permeants.

Pervaporation is a more practical and cost-effective method for recovering aroma compounds from a variety of agro-food products and their by-products than the current separation techniques (distillation and solvent extraction), which also require expensive materials and high energy usage [30]. When compared to traditional evaporative processes, pervaporation has a significant advantage due to its excellent solute selectivity, since aroma compounds can often be enriched several hundred times [31], [32].Furthermore, pervaporation is particularly suitable for recovering highly diluted solutes from processing streams because the separation process is based on distinct molecular interactions between the membrane

and the solute or the solvent with intended selective solute sorption, which represents a unique solution in the recovery of diverse aroma profiles [23], [33]

If the partitioning of target solutes toward the membrane is very favourable, especially when compared to the partitioning of the bulk solvent, then concentration depletion of these solutes at the feed-membrane interface may occur. This phenomenon can be reduced by providing particularly good external mass transfer conditions, which reduce the thickness of the mass transfer boundary layer at the membrane surface. This depletion phenomenon is named "concentration polarisation", even though it is simply a concentration boundary layer that can form in any chemical engineering process where the mass transfer occurs between two different contacting phases [30], [34]. Strategies for mitigating the mass transfer boundary layer impact typically include appropriate equipment design and operating conditions that ensure near-optimal fluid dynamic conditions [24].

Another membrane process which is very similar to pervaporation, although less affected by concentration polarisation, is vapour permeation [9]. This technique is a process very similar to pervaporation, the only difference being that the feed is not liquid, but a vapour. Vapour permeation may be used in two different ways for aroma recovery. In the first, a functional fermenter gas stream is directly fed to the membrane module. However, because aromatic compounds have a low partial pressure in the feed stream, a wide membrane area would be necessary because the driving force for mass transfer across the membrane would be insufficient. In the second, gas stripping, an industrially proven method for removing volatile organic compounds from water, may be used to create an aroma-rich gaseous feed stream at atmospheric pressure [9]. In this context, vapour permeation could represent an interesting alternative for recovering aroma compounds due to the adequacy of this technique for selectively separating aromas from diluted vapour streams, as already demonstrated [5].

In all of these processes (RO, PV and VP), the selectivity of target compounds towards water, the permeability to the target compounds, and the stability of the membrane under mild operational conditions employed are the most important parameters that define membrane performance. To meet market demands, there has been a major advancement in the development of new membrane materials with enhance transport capabilities in recent years [35]. Reverse osmosis, pervaporation and vapour permeation membranes comprise a dense polymer top layer with no visible pores, in which solute-membrane interaction occurs. These processes seem to be extremely distinct at first look, but a number of principles are common between them.

The transport model assumed for the pervaporation and vapour permeation processes is the solution-diffusion model In this model, the permeants solubilise in the membrane material and the permeants are transported through the membrane by a gradient of chemical

potential, or more simply by a gradient of concentration or partial pressure, between the feed and permeate membrane surfaces [26].

When compound *i* has a stronger affinity to the membrane than to the feed solution, it has a high sorption coefficient, S_i [-], meaning that it will sorb favourably at the membrane surface, resulting in a concentration gradient across the membrane. The compound then diffuses (the diffusivity of compound *i* is given by the diffusion coefficient in the membrane D_i [m²/s]) toward the downstream surface of the membrane as a result of the concentration gradient between the two membrane surfaces. Finally, at the downstream side, it is assumed that compound *i* instantly desorbs [24]. The transmembrane flux of *i*, J_i [mol/m²/s] is then given by equation 1.

$$J_i = \frac{L_i}{\delta} \cdot (C_{i,feed} - C_{i,perm}) \quad (1)$$

where L_i represents the permeability to compound *i*, δ is the membrane thickness and $C_{i,feed}$ and $C_{i,perm}$ are the concentrations of compound *i* in the bulk feed and in the permeate, respectively.

The permeability of a compound *i* is given by equation 2.

$$L_i = S_i \cdot D_i \quad (2)$$

where D_i and S_i represent the diffusion and solubility coefficients of compound *i*, respectively. The permeability to compound *i* can also be expressed as the flux normalized by the active, dense layer film thickness (δ) and the transmembrane pressure (Δ p), as shown in equation 3.

$$L_i = \frac{(J_i \cdot \delta)}{\Delta_p} \quad (3)$$

The selectivity of the membrane for compound *i* in relation to compound *j* is the ratio of the permeabilities to the different compounds as described by equation 4.

$$\alpha_{i,j} = \frac{L_i}{L_j} \quad (4)$$

When comparing distinct processes, one should compare the intrinsic transport properties of the membranes under consideration independently from the operating conditions (feed composition, feed stream fluid dynamics, and permeate pressure). In contrast to fluxes, permeability and selectivity are intrinsic transport parameters [24].

1.1.2. Modelling fractionated condensation for off-flavour removal

Vapours might be selectively condensed during a condensation process, which can be performed at different temperatures in an in-series condenser system to achieve fractionated condensation [23]. Fractionated condensation enables aroma fractionation for separation between different groups of aromas (or for off-flavour removal), concentrating the aroma compounds [10]. By using the operating conditions of permeate pressure and condensation temperature applied in a 1st condenser it is possible to estimate the condensation of target aromas and achieve fractionated condensation [36]. However, between compounds with very similar thermodynamic properties it might be very challenging to achieve a target compound separation.

The high energy costs associated with the condensation process (total or fractional) are one of the key issues for competitiveness. However, when condensation is integrated with pervaporation/vapour permeation processes, with lower vapour flow rates, it becomes more economically viable [23].

In practice, hybrid separation techniques are becoming increasingly important as they are more efficient than the implementation of homogenous separation units for complex separation problems. The industry will take advantage of hybrid processes with lower costs, reduced energy needs and higher product efficiency to achieve sustainable processing [37]. Rigorous modelling is required for developing hybrid separation processes properly [38].

Baudot & Marin [17] suggested a quantitative model for the recovery of highly diluted aroma compounds in aqueous feed solutions by pervaporation with two-stage condensation. This model allowed the permeate stream to be characterized under variable temperature, permeate pressure and upstream conditions after the condensation. It was observed that considering thermodynamic liquid-vapour equilibrium in the condenser, inert gases may be present in the downstream circuit due to minor leaks in the installation. Following this work, Brazinha *et al.* [10] developed a semi-empirical model which enabled the prediction and modelling of the condenser operation conditions not only in condensers in-series system but also in complex feed streams with the presence of strong co-solvents and dissolved non-condensable gases, usually generated in fermentation systems. In this work, this model was applied to aqueous model systems with different chemical families, to study the separation of off-flavours during the aroma recovery process. After validation in simpler systems, it was implemented in hydroalcoholic systems with non-condensable gases.

This PhD research project aims to study the aroma recovery by membrane processes and fractionated condensation of natural valuable aromas. This thesis is divided in two main sections: a first section is focused on the recovery of seafood aromas from the canning industry effluents, resulting from the fish cooking step; and the second one refers to the recovery

of wine aromas, produced during the vinification process, which are loss by venting from the headspace, being a vapour effluent.

1.1.3. Seafood aromas case-study

The seafood cannery industry represents 14.4% of the total fish market, with tuna, sardine, sardine-type and mackerel as the main canned fish species. Canned fish is considered an intrinsically healthy, convenient and tasty food [39], [40]. Although the industry is in a downward trend due to the reduction of catch shares, it remains an important industry, particularly in Portugal, representing 8% (74 133 tonnes) of the world's production of canned sardines. 20 fish canning facilities in Portugal produce around 44 thousand tonnes of canned products each year [41], [42].

Wastewater generated through salting, washing, cooking and washing steps represents 10, 35, 15 and 40% of the total canning effluents, respectively. Currently, these streams are not valorised, representing the main environmental problem of seafood cannery industries. Cooking is an essential operation that produces an abundant rich effluent known as "cooking juice" or "cooking wastewater" [39], [43]. Seafood cooking wastewater can be characterized as nontoxic, with a high and variable content of organic matter, both as suspended and dissolved solids. Fish and shellfish canning industries generate different liquid effluents that present a wide range of characteristics according to the raw materials processed. The treatment of these effluents is particularly difficult due to the high content of organic matter, salts and the significant amounts of oil and grease they comprise [43], [44].

Due to its simplicity and efficacy, the coagulation-flocculation process is recognized as one of the most essential and commonly used industrial wastewater treatment procedures [45]. This procedure consists of two independent steps: (1) vigorous agitation for quick mixing of dispersed coagulant with water/wastewater to be treated, and (2) flocculation for agglomeration of tiny particles into well-defined flocs. Finally, the flocs settle and are removed as sludge, while the cleaned water/wastewater (supernatant) is transported to a later treatment procedure or discharged into a waterway [46]. It is a versatile process, which can be combined as a pre-treatment, post-treatment, or even the main treatment of wastewater, depending on the constitution of the mixture [47]. Usually, this is carried out using iron, aluminium salts or other non-food grade chemicals, which indeed are efficient to clean the water, but represent one limitation to this process, due to toxicity and health hazard posed by inorganic coagulants. To enable the use of the sludge for food/ feed purposes, some polysaccharides such as carrageenan, alginate, and carboxy methylcellulose were proposed as food-grade coagulant/flocculant agents [46], [47].

Seafood aromas are combinations of low molecular weight, volatile, organic and sometimes non-polar compounds. The aromas are predominantly alcohols, aldehydes and ketones, and nitrogen and sulphur-containing substances, which include some off-flavour

components [20], [48]. Aldehydes are the major product of lipid autoxidation and thermal degradation of fatty acids. These molecules play an important role in the aroma of foods and may be responsible for undesirable oxidized aromas. Alcohols make a minor contribution to the flavour unless they are present in high concentrations. Ketones constitute a minor fraction formed by thermal degradation and oxidation of polyunsaturated fatty acids. They are assumed to contribute to the floral and fruity aroma of seafood products [49]. During the industrial processing of seafood, the aromatic compounds are subjected to transformations, such as oxidations via chemical or microbial pathways. These reactions generate sulphur-containing volatile compounds, which change the overall seafood odour from desirable to unpleasant [50].

Some studies focused on the aroma recovery of seafood by membrane processing were performed with different matrixes. Different methods can be used for the treatment of seafood cooking wastewaters, which can be divided into physicochemical methods (centrifugation, coagulation/flocculation, dissolved air flotation and activated carbon adsorption), membrane processes (reverse osmosis (RO), microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and pervaporation (PV)) and biological treatments [21], [40], [51]. New processes based on the combination of membrane operations and conventional separation technologies give interesting benefits that cannot be achieved when developed as one concept and offer new opportunities in terms of competitiveness, quality improvement, process or product novelty and environmental friendliness [18]. **Table 1.1**. presents a summary of different studies published on aroma recovery from seafood cooking wastewaters. To the best of our knowledge, this is the first study focused on sardine cooking wastewaters.

Table 1.1. Aroma recovery from seafood cooking wastewaters by membrane technologies.

Effluent	Process	Conclusions	Ref.
Tuna, shrimps and buckies cooking wastewaters	NF or RO after pre-treatment by UF	RO was more efficient to retain all aroma compounds than NF, but UF pre-treatment was needed to maintain acceptable RO permeation fluxes. Better retention by RO for shrimps and buckies cooking wastewaters than for tuna	[52]
Shrimp cooking wastewaters	Membrane process integrating electrodialysis (ED) and RO	Aroma concentration by a membrane process integrating ED and RO seems economically feasible, with a pay-back time inferior to three years.	[53]
Tuna cooking wastewaters	NF and MF+NF	The two-step process of MF pre-treatment before NF concentration allows a concentration factor of 5 with good permeation fluxes.	[54]

Brown crab boiling wastewaters	PV (Pervap 4060, PDMS)	PV can successfully concentrate the aroma compounds from the real boiling effluents with enrichment factors in the range of 1-20.	[48]
Snow crab cooking effluents	RO spiral-wound polyamide membrane	The cooking effluents generated can be concentrated and turned into a natural aroma for the food industry. The chemical composition of the concentrates was mainly proteins, minerals and desirable aroma compounds.	[49]
Oyster cooking wastewaters	PV (PDMS membrane)	The main aroma compounds of oyster cooking wastewaters could be successfully concentrated by pervaporation, and an enrichment factor of 35 was reached for 1- octen-3-ol at permeate pressure 1750 Pa and 30 °C.	[55]
Mussel cooking wastewaters	ED+RO+NF	Aromas were recovered by reverse osmosis with prior desalting by electrodialysis. The integrated system allows a concentration factor of 5.	[56]
Lobster cooking wastewater	RO (CF of 5)	The concentrate obtained, mainly composed of minerals (60.4%), proteins (30.0%), and desirable aroma compounds, was safe for consumption and exhibited desirable properties. However, variability in the cooking effluent composition between batches must be reduced to ensure consistency in the finished product.	[57]

1.1.4. Red wine aromas case-study

Wine production is one of the most important agricultural activities throughout the world, especially in Portugal, with a year of wine production of around 650000 m^3, of which 425000 m^3 is red wine [58]. Wine is a complex blend of hundreds of chemicals, the majority of which contribute to sensory traits including colour, texture, and fragrance. There is great interest in wine aroma, and several components have been recognized as playing a role in various sensory attributes [59].

In the case of wine, the aroma compounds occur from several sources: i) directly from the grape berry; 2) from yeast and bacterial metabolism; 3) from the extraction of heat-treated oak wood; 4) from chemical reactions upon storage and 5) from enzymatic and/or chemical reactions of non-volatile precursors during grape processing, winemaking and/or storage [60]. The aromatic profile is generally considered to be one of the most individual characteristics of a wine. However, aroma compounds are lost during grape must fermentation owing to escaping carbon dioxide sweeping, which ultimately affects the wine's aroma [61].

During the vinification process, the aroma profile may change significantly throughout processing owing to chemical and physical changes in the aroma complex. Chemical changes

such as oxidations or Maillard reactions can occur during heat treatment, resulting in the loss or biosynthesis of new aromas. Physical changes in the aroma composition can also occur after excess water concentration, and some volatile compounds, such as esters, due to evaporation. These variations in aroma profile are regarded as unpleasant, and several strategies can be used to prevent or reduce adverse changes in off-flavours composition. Separation processes, such as distillation or pervaporation/vapour permeation, use physical properties of aromas such as solubility, relative volatility, and hydrophobicity [62].

A large proportion of volatile components can be lost through the fermenter venting during vinification, due to their inherent volatility and the ease with which they are loss associated with the release of carbon dioxide. Temperature, the rate of gas evolution, and the type of fermentation vessel being used all affect how much aroma compounds are lost. Ethanol concentration and carbon dioxide evolution both have an impact on the concentration of aromas in the vapour phase. These conditions influence how the wine volatiles are released and carried out from the headspace of the fermentation vessel, in addition to the impact of temperature [61].

The headspace generated during the vinification process has already been analysed and shown to be mostly composed of esters and alcohols, which are thought to be especially significant for wine aroma. Esters (such as ethyl acetate, isoamyl acetate and ethyl hexanoate) are thought to be important components of fruity taste [61], [63]. Ferreira *et al* [64] estimated that the amount of aroma compounds lost related to the CO_2 stripping effect represents up to 84% of the total amount of higher ethyl ester produced and more than 50% for the remaining compounds.

Regarding the desirable aromas, there are several approaches to promote aroma recovery, aiming to minimize aroma loss. This can be done by generating an aroma concentration that can be added back to the finished product, therefore improving its sensory quality [62]. Some separation methods such as condensation, absorption and adsorption (e.g. on activated carbon) are disadvantageous in terms of economic and energy intensity and are limited in their efficiency [65].

The presence of an off-flavour in an alcoholic beverage can lead to a consumer's perception of inferior quality and negative publicity, which can be extremely costly to the industry. Preventive actions are focused on their hygienic nature, such as sanitized front-end processing equipment, whereas the remediation approach can be divided into two groups, those aimed at lowering headspace off-flavour concentrations and those aimed at removing off-flavours. Despite decades of study, the most significant difficulty for brewers and wineries is the degradation of aromas, particularly the loss of fresh aromas and the emergence of off-flavours [66].

Focusing on the off-flavours, the presence of volatile phenols in red wines, namely 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG), has a detrimental impact on the aroma classified as "Band-aid," "medicinal," "barnyard," and "stable," as well as "metallic" taste descriptors [67]. The most significant organisms responsible for the generation of these aromas in wine are yeasts categorized as *Dekkera*/*Brettanomyces* spp. When wine is produced or stored in oak barrels, *Brettanomyces* spoilage is common. Because yeast develops slowly, smells are generally only imparted when the wine is matured, near the end of its cycle of usage (5–6 years). While *Brettanomyces* is present in low quantities early in fermentation, it may be unnoticed because it is outnumbered by other indigenous yeast. High concentrations of 4-EP and 4-EG were identified in all wines, with a poor influence on the product's quality, most likely owing to the presence of contaminating organisms in the barrels, which were old [59].

The off-flavour reduction or aroma recovery from wine by membrane processing has been studied. In **Table 1.2**., different techniques reported for aroma recovery from wine are summarized.

Table 1.2. Aroma recovery from wine by membrane technologies

Feed	Process	Conclusions	Ref.
Red Wine	Adsorption (XAD-16HP) +Reverse Osmosis	The integrated process proved reliable and effective in the reduction of off-flavours from red wine: it lowered the concentrations of 4-EP and 4-EG and partially adsorbed a series of herbaceous and “green”-associated C6 alcohols	[68]
Wine-must fermentation	Organophilic pervaporation (POMS)	Pervaporation can be highly suitable for the continuous recovery of very complex and delicate aromatic profiles produced during microbial fermentation	[69]
Wine model solution	Organophilic PV (POMS) + fractionated condensation	The model developed for the recovery and fractionation of aromas by organophilic pervaporation/fractionated condensation proved to be a useful tool for predicting the recovery of each permeating species in each condenser	[10]
Model solution of alcoholic beverages	Pervaporation (PDMS-CA)	The coupling effect between ethanol and aroma compounds on their permeability was studied. It was concluded that the presence of aroma compounds decreased the permeability of ethanol to some extent, while it had a small effect on the water permeability. The ethanol concentration in the feed affected differently the mass transfer of each compound.	[70]

Model solution of ethyl acetate	Gas-stripping + vapour permeation (PDMS)	The stripping rate increased with the gas flow rate. Vapour permeation using PDMS membranes was shown to be rather appropriate for selectively recovering the stripped aroma compound.	[9]
Apple Cider beverages	Gas-stripping + condensation	Removal of ethanol and other aromas by recirculated CO_2, followed by recovery from the gas stream by condensation offers several advantages including the use of a "natural", abundant, and essentially free extractant, without application of heat to the beverage.	[71]
Red wine	Gas-stripping + condensation	The condensate is a mixture of water, ethanol and a significant amount of aromas (1200 mg/L). Recovered compounds were mainly alcohols and esters of secondary origin, which have a significant impact on wine aroma.	[61]
Wine must model solution	Sweeping gas pervaporation + absorption in membrane contactors	This system allows for the capture of aroma compounds present in the pervaporation vapour permeate stream by absorption, avoiding the need for energetically intensive vacuum conditions and condensation steps. This concept can be applied for aroma recovery from headspaces and off-gas streams of fermentative processes.	[6]

1.2. Research Strategy and Objectives

During this PhD project, two national research projects entitled "MobFood" and "Multi-BioRefinery" were developed. They had as a common objective the promotion of the Portuguese bioeconomy through the valorisation of food industry by-products, where our role was focused on the recovery and fractionation of natural aromas/flavours depleted in off-flavours. Both projects were carried out in collaboration with a partnership with the company A Poveira, S.A., a fish canning industry.

The research strategy of this PhD project comprises the recovery of natural flavours with off-flavour removal by integrated membrane processing and fractionated condensation of two different case-studies: 1. Sardine cooking wastewaters/seafood model solution and; 2. red wine model headspaces. These case-studies contribute most significantly to the regeneration of these economically important industries, relevant in Portugal and worldwide, promoting a circular economy approach towards zero waste. The goal is the design of integrated systems to recover natural aromas comprising the (i) recovery and fractionation of natural aromas and (ii) assure the removal of off-flavours, so the recovered aromas could be delivered in final food products, as natural flavourings. **Figure 1.1** shows the main processing steps explored in this PhD work.

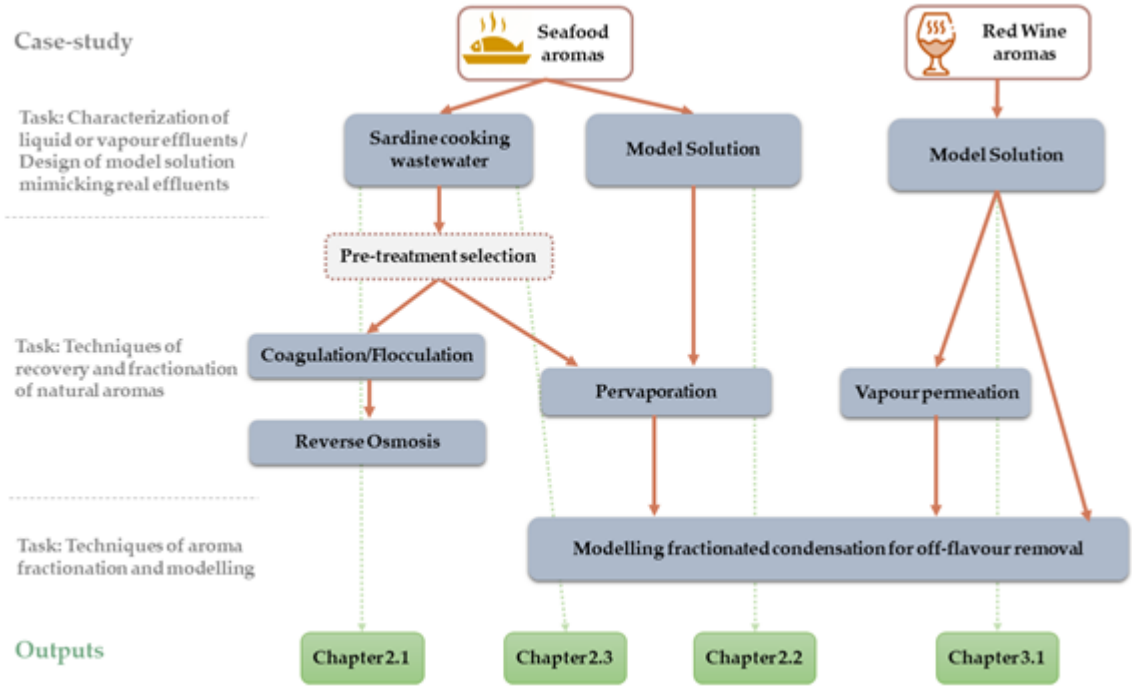


Figure 1.1. Schematic representation of the various tasks involved in this PhD work

The first task was focused on the characterisation of effluents and the design of model solutions applied to both case studies included in this PhD project. This characterisation of the effluents and corresponding aroma fractions was performed in terms of their chemical composition. For the case study of seafood aromas, the real solution used was sardine cooking wastewaters and the model solution designed included aromas of fish, oysters and shrimps, for a more comprehensive application. For the red wine aromas, a model solution was designed considering the most common esters present in red wines and the off-flavours generated during the vinification process. For both model solutions, the concentration of the compounds of the model solutions was intended to mimic real conditions.

The next phase was focused on the aroma recovery processes. In the seafood aromas case study, due to the heterogeneity of the sardine cooking wastewaters, different types of pre-treatments were studied: the addition of an antioxidant extract for aroma conservation, prompted by the easy aroma degradation, and a coagulation/flocculation step for sample clarification. After the optimisation of the coagulation/flocculation process, a process of reverse osmosis was applied to concentrate the aromas, due to the presence of highly diluted aroma compounds in the aqueous solution. Applying the seafood model solution, the effect of permeate pressure in organophilic pervaporation was studied for aroma recovery, followed by fractionated condensation. In red wine aromas, vapour-permeation combined with fractionated condensation was studied for aroma recovery.

To achieve an off-flavour removal, a mathematical model was applied and validated for two condensers-in-series, which assumes a stage of liquid–vapour equilibrium in each

condenser. After studying the total condensation by recovering all vapours of each effluent, the model was applied to a two-step condensation and predict the production of aroma fractions with the aimed characteristics. The effect of the operating parameters of pressure and temperature in each stage of the fractionated condensation process was assessed when using two condensers-in-series. The model was validated for model (for both case studies) and real solutions (for the seafood case-study) to understand the effect of the matrix complexity. For the seafood case-study, pervaporation-fractionated condensation was studied, aiming for an off-flavour removal. In the red wine case study, an integrated vapour permeation-fractionated condensation system was compared with a fractionated condensation, as a stand-alone step, to study the fractionation of off-flavours from the target aromas.

Therefore, the main objectives of this project are:

- Design of integrated systems for the valorisation of aromas from liquid and vapour effluents, employing membrane processes (reverse osmosis, pervaporation or vapour permeation) that operate under mild operation conditions, to produce desirable aroma concentrates, free from off-flavours, to be applied as natural flavouring additives for feed and food applications;
- Test and validate a mathematical model for predicting the partial condensation of a complex vapour (including the effect of the co-solvent ethanol and non-condensable gases on the recovery of aroma compounds) with different chemical families for off-flavours separation;
- Demonstrate the applicability of the mathematical model to i) different case-studies - seafood aromas with model solutions and real solutions (involving the effect of the presence of high lipid and protein content) - and ii) different processes: fractionated condensation and combined vapour permeation/fractionated condensation, to recover red wine aromas free from off-flavours;

1.3. Thesis Outline

The work performed during this PhD is organised in the following way: a first chapter where the thesis background, motivation and outline are presented, followed by two chapters that correspond to scientific original papers accepted or submitted for publication, which describe the experimental work performed and the results obtained, and finally, a chapter that presents the overall conclusions of the work and suggestions for future work.

A summary of the content of each chapter is described below:

Chapter 1: Background and Motivation – Describes the background and motivation for this PhD, the main objectives and the thesis outline.

Chapter 2: Integrated systems for recovery of seafood aromas - Describes the design of two different integrated systems for the production of seafood aromas. **Chapter 2.1** reports the optimization of the flocculation/coagulation pre-treatment, necessary due to the high heterogeneous composition of sardine cooking wastewaters. Additionally, it contains the design of an integrated flocculation/reverse osmosis process, which allows for the recovery of proteins, lipids and aromas, reducing the environmental impact of these effluents and the water consumption of this industry. **Chapter 2.2** describes the design of an integrated organophilic pervaporation/fractionated condensation process to recover valuable aromas from aqueous effluents of seafood processing, assuring that the aromas recovered are (as much as possible) free from off-flavours to apply as seafood flavouring additives. The condensation process was assessed, with the support of a developed thermodynamic model, which allows for simulation of the fractional condensation process and selecting the optimal operating conditions for a given target separation, with a minimum of experimental tests and reliable thermodynamic properties. Finally, **Chapter 2.3** demonstrates the validation of the thermodynamic model developed **(chapter 2.2)** with sardine cooking wastewaters.

Chapter 3: Integrated system for production of red wine aromas - details the design of the fractionated condensation, as a stand-alone step or in an integrated vapour permeation-fractionated condensation system with a CO_2 stripping effect. The optimal operating conditions selected from the thermodynamic model developed for an off-flavour separation **(chapter 2.2),** were applied to more complex feedstocks, such as non-aqueous media with the presence of non-condensable gases.

Chapter 4 comprises the conclusions and suggestions for future research on the production of desirable aromas.

2.
INTEGRATED SYSTEMS FOR THE PRODUCTION OF SEAFOOD AROMAS

2.1. Clean technologies for production of valuable fractions from sardine cooking wastewaters: an integrated process of flocculation and reverse osmosis

Published as: M. João Pereira, Oceane Grosjean, Manuela Pintado, Carla Brazinha and João Crespo, "Clean technologies for production of valuable fractions from sardine cooking wastewaters: an integrated process of flocculation and reverse osmosis", Clean Technologies (2022), 4, 276-295.

The author was involved in planning and performing the experiments, as well as in the discussion and interpretation of the results and preparation of the manuscript.

2.1.1. Summary

The increase of 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 the 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, that 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 re-used in the overall process (e.g. as water for washings) or discharged at a lower cost, with environmental benefits and economic savings.

2.1.2. 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% of agro-food wastes until 2030, which may be achieved by reducing pollutant discharge from industrial operations and reuse and valorisation of wastes and effluents [43]. 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 [45] 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% (74133 tonnes) of the world's production of canned sardines, from which the top 20 fish canning facilities produce around 44 thousand tonnes of canned products each year [41], [42]. The canning industry generates a large amount of wastewater, which is particularly difficult to treat due to its high content of salt organic matter (lipids and proteins) [44], [51].

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 re-used in the overall process (the preferable option) or to be discharged. The European Directive 98/83/EC allows water reuse in the industrial process [21]. This approach brings further revenues to the seafood processing industries, both by the added-value food components [45] and by the reduction of 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 [51], [72].

Membrane separation processes may efficiently recover high-value, sensitive compounds, from seafood wastewater as these processes may operate under mild operating conditions, e.g. at mild temperatures. In particular, reverse osmosis may also produce water

from effluents, to be re-used in the overall process and is suitable to process 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 re-used (depending on the feed concentration) [2], [20].

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 [21], [52], [73].

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 wastewater by coagulation/flocculation for feed/food applications demands the use of food-grade coagulants and flocculants [72]. The natural polymer chitosan is a suitable coagulant [74] 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 to clean the water, but would represent a limitation to this process, due to toxicity and health hazard 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 [46], [75].

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 pretreatment 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* [45] recover 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. [49], [57] 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, treat and reduce the discharge of the effluent with environmental and economic benefits. Following this approach is aimed the production of: i) a solid fraction with a 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) reverse osmosis permeate with a minimised COD value (organic load).

2.1.3. Material and Methods

2.1.3.1. 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, 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 FILMTEC™ Flat Sheet SW30 HR (Dow Filmtec, USA). A polyester support web, a microporous polysulfone interlayer, and an ultra-thin polyamide barrier layer on the top surface compose this thin-film composite membrane. The effective area of the membrane was 5.1×10^{-3} m^2.

2.1.3.2. Experimental procedure

At the moment of sampling the sardine cooking wastewater, at the outlet of cooking chambers, a 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 2.1**. describes the flow of the experimental studies carried out in this work.

To produce valuable fractions from sardine cooking wastewater, coagulation/flocculation pre-treatment was applied before reverse osmosis

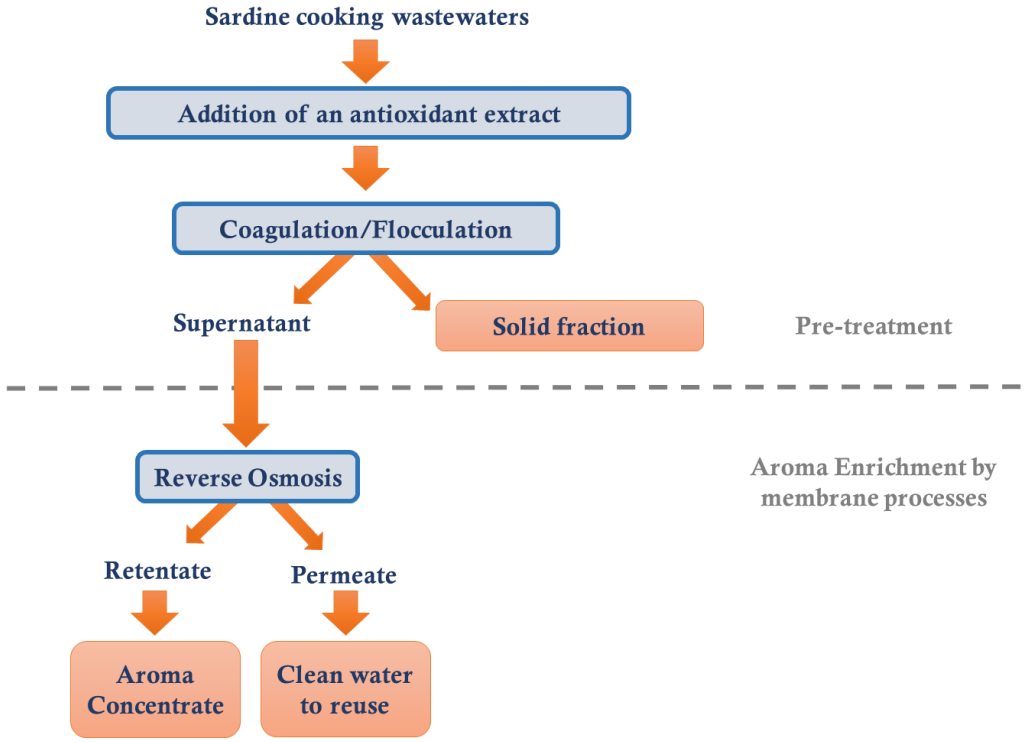


Figure 2.1. Flow diagram summarizing the experimental studies performed in this work.

Each processing step of the integrated process of coagulation-flocculation and reverse osmosis (see **Figure 2.1**) 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 at 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 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). It also evaluated the impact of the pre-treatment process on membrane performance (membrane permeance). 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.

2.1.3.2.1. 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 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 set-up of this pre-treatment is explained in **Figure 2.2**. 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 minutes at 500 rpm at room temperature using a stirrer (OHS 100 digital, Velp Scientifica, Italy). In the last 10 seconds of high stirring, the flocculant agent was added and the stirring velocity was down to 40 rpm for 30 minutes. In the end, the sample was centrifugated (Marathon 22 KBR, Fischer Scientific, New Hampshire, EUA) at 3800 x *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.

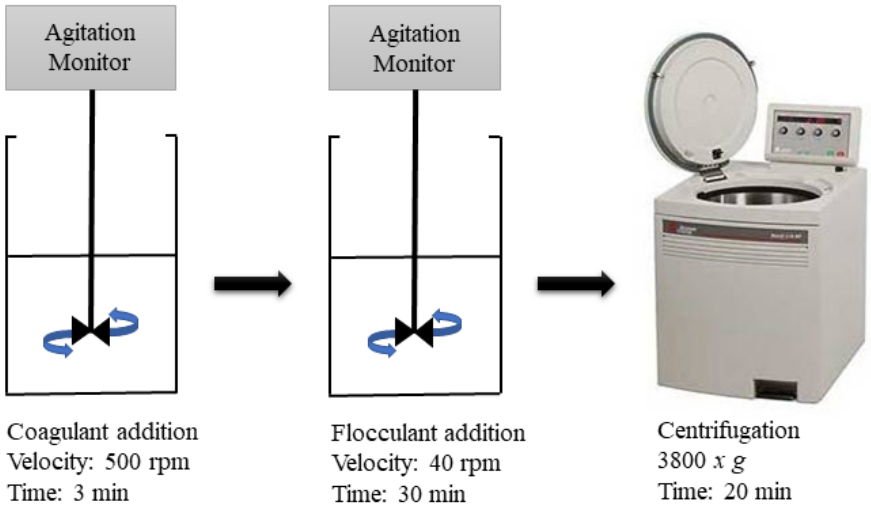


Figure 2.2. Schematic representation of the coagulation/flocculation process

2.1.3.2.2. Reverse Osmosis (RO)

The reverse osmosis 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 x 10^{-3} m^2 was used under controlled transmembrane pressure conditions (EVONIK METCell, Evonik, United Kingdom, see **Figure 2.3**). A porous stainless-steel disc supports 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.

The protocol of 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 minutes, followed by alkaline treatment with 6 g/L NaOH in total recirculation mode for 30 minutes. After these stages, the membrane was rinsed with water until the neutrality of the streams and the water flux were determined to evaluate the efficiency of the cleaning procedure. The membrane permeability after the cleaning procedure was restored (with at least 91% of permeability recovery).

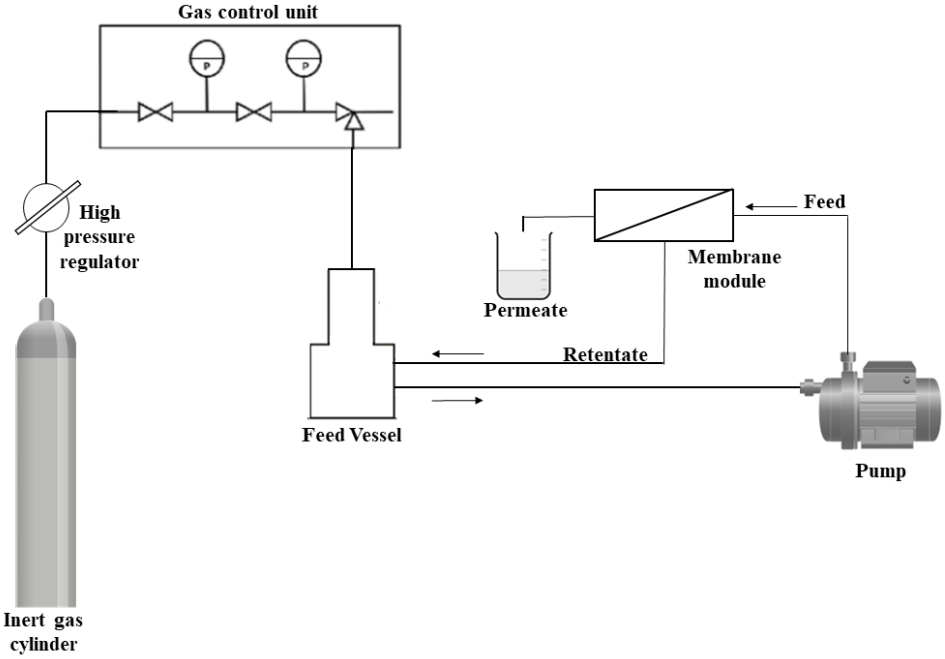


Figure 2.3. Experimental reverse osmosis setup using EVONIK METcell unit

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 5:

$$J = \frac{Q_P}{A} \tag{5}$$

where Q_P is the permeate flow rate (L/h) and A is the surface area of the membrane (m^2). The permeance of the membrane is given by Darcy's relation [25]:

$$L_p = \frac{Q_P}{A \times P} \quad (6)$$

where Lp is the permeance ($L.h^{-1}.m^{-2}.bar^{-1}$), 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:

$$VCF = \frac{Volume_{initial}}{Volume_{retentate}} \quad (7)$$

2.1.3.2.3. Analytical methods

Chemical Oxygen Demand (COD) Measurement

COD is the amount of oxygen required by a chemical oxidizing agent (typically chromic acid) to oxidize 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, Germany, Dusseldorf) was used and the absorbance was measured with a HACH DR3900 Spectrophotometer (Hach Lange GMBH, Germany, Dusseldorf). For some samples, a dilution was required and distilled water was used for that purpose.

Total protein content

The Lowry method was used to determine the protein content [76], [77]. 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, 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.

Total lipid content

The total lipids were determined using Bligh & Dyer method [78]. 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 shaken again for 2 minutes. 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 weighted to determine the lipids content of the sample.

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 x 0.25 mm i.d. x 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 a 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.

2.1.4. Results

2.1.4.1. Characterization of Sardine Cooking Wastewater

The sardine cooking wastewater varies depending on the overall output of the fish canning industry. To obtain a representative dataset of wastewater characteristics, several samples (6 samplings taken at different times of the year) were collected and analysed. Their characteristics are shown in **Table 2.1**.

Table 2.1. Characterization of sardine cooking wastewaters

Parameter	
pH	6.5 ± 0.1
COD (mg/L)	28080 ± 100
TSS (%)	4.08 ± 0.26
Total protein content (mg/ml)	25.38 ± 1.95
Total lipids content (g/100g)	28.13 ± 2.84

Table 2.2. 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 wastewater [79], [80]. 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 [81].

Table 2.2. 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
Octa-1,5-dien-3-ol, (5Z)-	6.50%	
2-Ethylhexanol	0.57%	
1-Octanol	44.49%	
Ketones		
2-Nonanone	3.38%	0.001
3,5-Octadien-2-one	8.47%	
3,5-octadien-2-one, (3E,5E)-	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. The aromas were identified by comparing their retention indices relative to C8–C20 n-alkanes and mass spectra to those in the NIST Library Database. Quantification was done with calibration curves of the pure standards, evaluated under the same circumstances. 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 wastewater 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 [79]. 2-penten-1-ol and 1-octen-3-ol were important alcohols in the fresh sardine aroma profile [81], [82]. 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 3 aromas, in particular, were identified in all samplings [81]. 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.

2.1.4.1.1. 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 2.4**.), to ensure a minimum deterioration of aromas by oxidation.

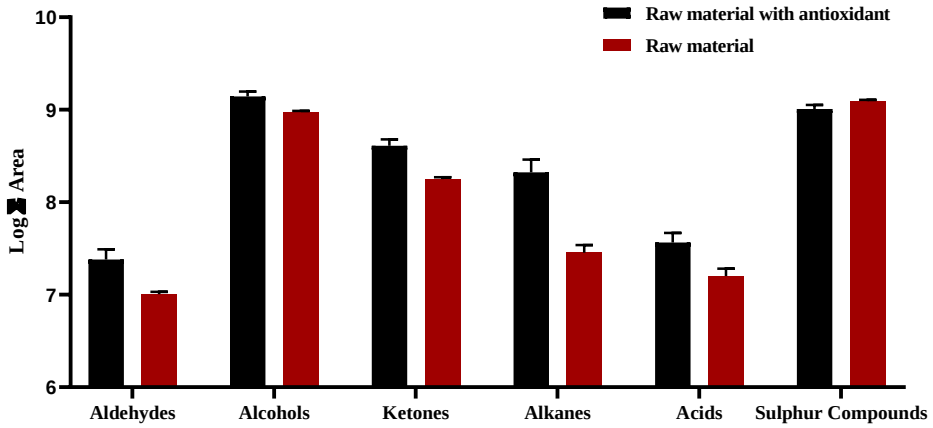


Figure 2.4. Effect of addition of antioxidant extract on the different classes of aroma compounds identified in sardine condensates

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).

2.1.4.2. 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 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 [83], [84].

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 2.3**).

Table 2.3 - 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.	4.60	35.97±2.1	56.02±0.5
	Supernatant	95.40		
Carrageenan (10 mg/L)	Solid F.	4.35	41.77±0.3	54.50±4.0
	Supernatant	95.65		
Alginate (10 mg/L)	Solid F.	4.46	42.32±0.8	40.22±1.5
	Supernatant	95.54		

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 [46]. 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 [46] 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 [74], [85], [86].

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 2.4**).

Table 2.4. 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/100g)	Protein recovery in solid fraction (%)	Lipid recovery in solid fraction (%)
100/10*	Solid F.	4.66	30.33±2.84	25.26±0.92	**79±1**	**64±4**
	Supernatant	95.34	5.44±0.30	10.16±1.73		
200/20	Solid F.	10.41	21.19±1.59	20.02±2.50	77±2	68±3
	Supernatant	89.60	5.92±0.55	9.08±1.08		
300/30	Solid F.	6.34	24.70±0.29	26.11±0.44	73±1	55±2
	Supernatant	93.66	6.80±0.29	10.63±3.45		
400/40	Solid F.	5.38	27.55±1.86	18.99±2.26	75±1	65±7
	Supernatant	94.62	6.47±0.13	9.35±2.17		
500/50	Solid F.	10.39	16.83±1.38	7.06±1.21	73±2	72±4
	Supernatant	89.61	6.87±0.39	7.77±1.51		
600/60	Solid F.	8.16	25.12±2.30	16.78±1.44	**71±2**	**82±1**
	Supernatant	91.84	7.28±0.57	5.13±0.06		

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 similarly.

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 protein 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 [85], 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 a 90% protein recovery with the chitosan/carrageenan combination. Forghani *et al*. [45] 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 [74], [85], [86], 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 2.3**.), 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 2.5**).

Table 2.5. 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/100g)	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 similarly.

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*. [86] 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 2.5**. presents the results for the chitosan/carrageenan combination.

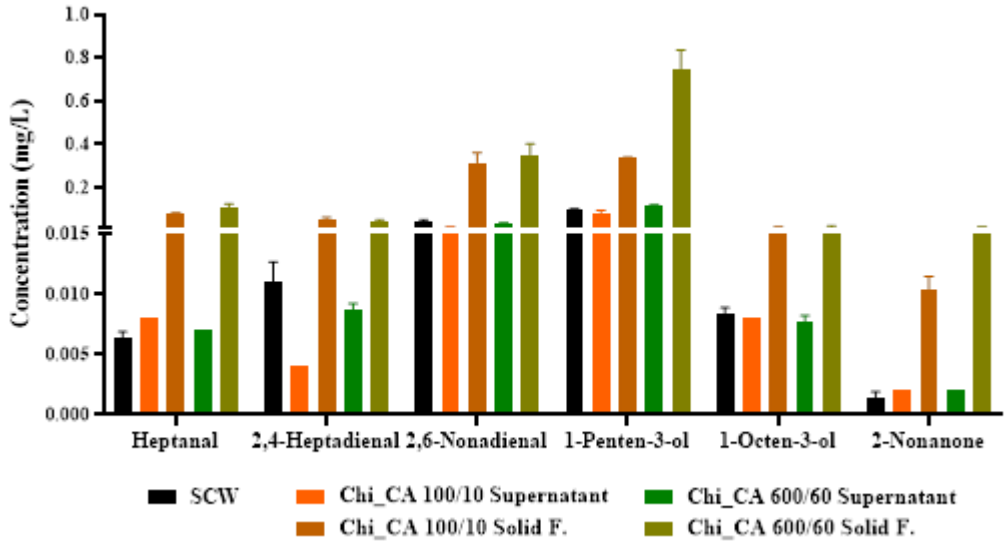


Figure 2.5. Concentration of the different aromatic markers present in the two fractions obtained by coagulation/flocculation using chitosan/carrageenan. SCW-sardine cooking wastewater

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 [87]. 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.* [88] 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 2.6**.

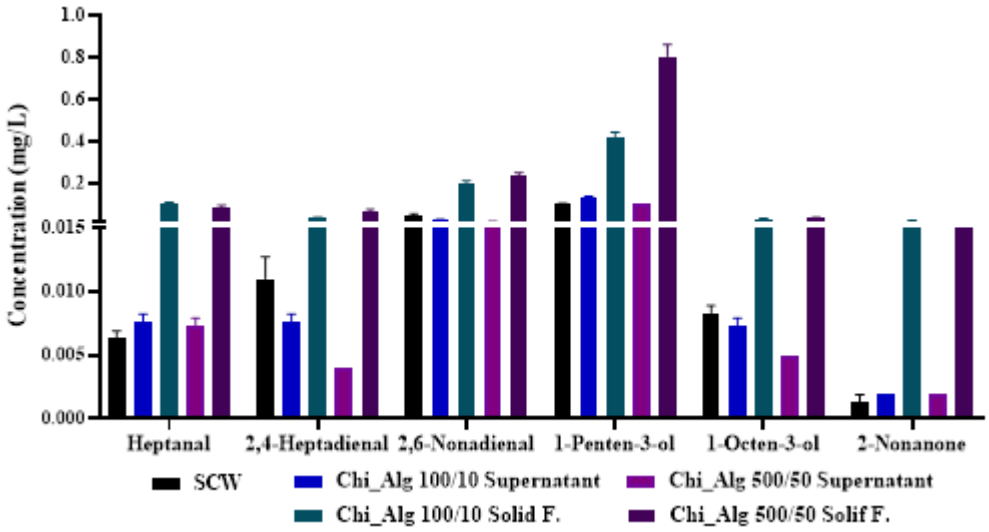


Figure 2.6. Concentration of the different aromatic markers present in the two fractions obtained by coagulation/flocculation using chitosan/alginate. SCW-sardine cooking wastewaters.

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.* [89] whose study shows an anti-cholesterol effect of chitosan in rabbits and broilers.

To obtain a safe quality ingredient, the effect of an additional sterilization step on the texture and aroma content of the solid fractions was evaluated (**Figure 2.7**.). 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.

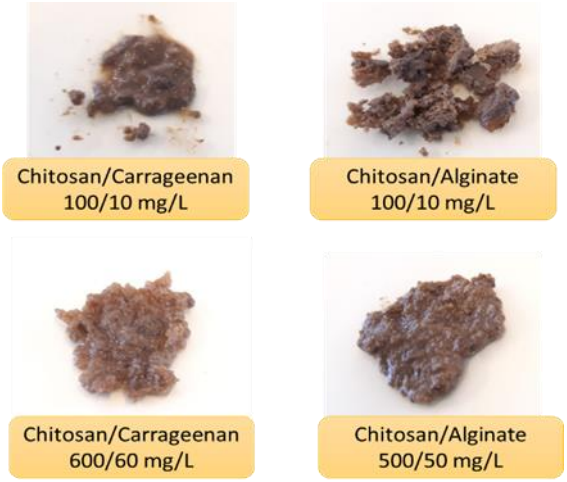


Figure 2.7. Visual aspect of the selected solid fractions after sterilization

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. 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 2.8**.).

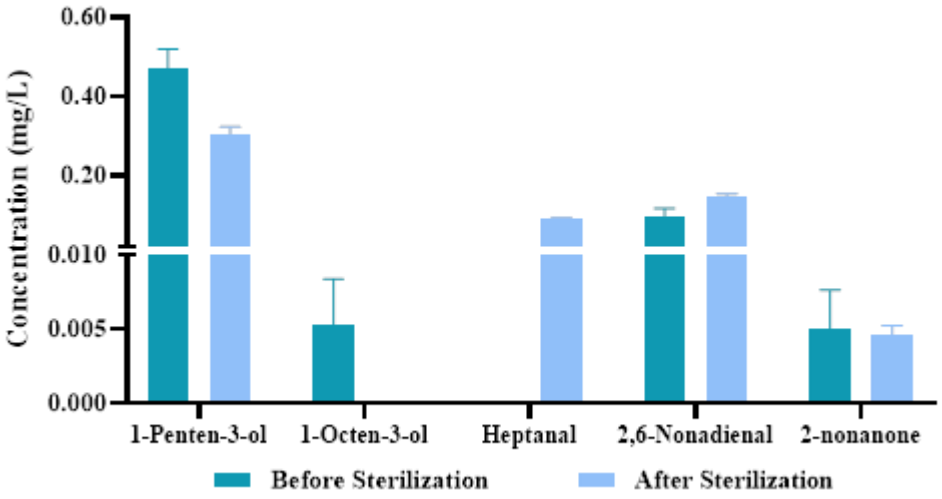


Figure 2.8. Effect of sterilization on the concentration of aromas presents in the product obtained with the chitosan/carrageenan combination at higher concentration.

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 [90]. In future studies, the impact of non-thermal sterilization methods should be evaluated, e.g. 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 to understand the effect of this pre-treatment on the COD values of the supernatants (**Figure 2.9**.).

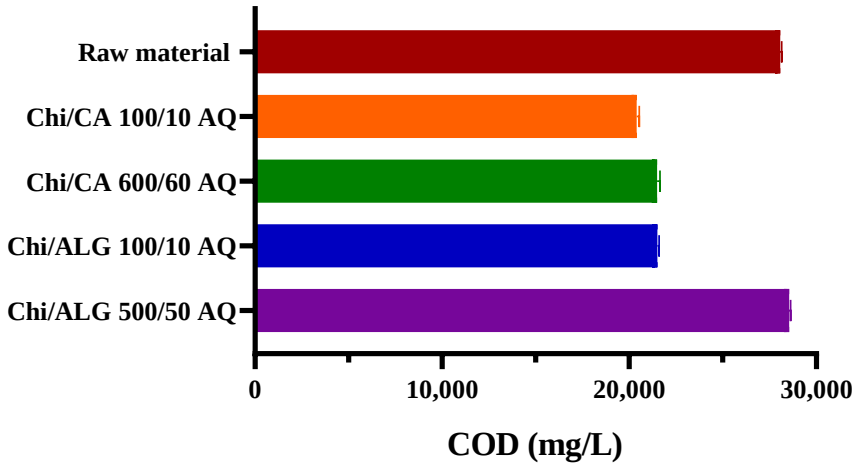


Figure 2.9. Effect of the coagulation/flocculation process on the COD levels of the supernatants. Note: Legislation allows for effluent discharge for COD values <2000 mg/L O_2

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% concerning 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.

2.1.4.3. 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 2.10**. shows the permeance data (permeate volume obtained divided by the driving force applied) plotted against the volumetric concentration factor.

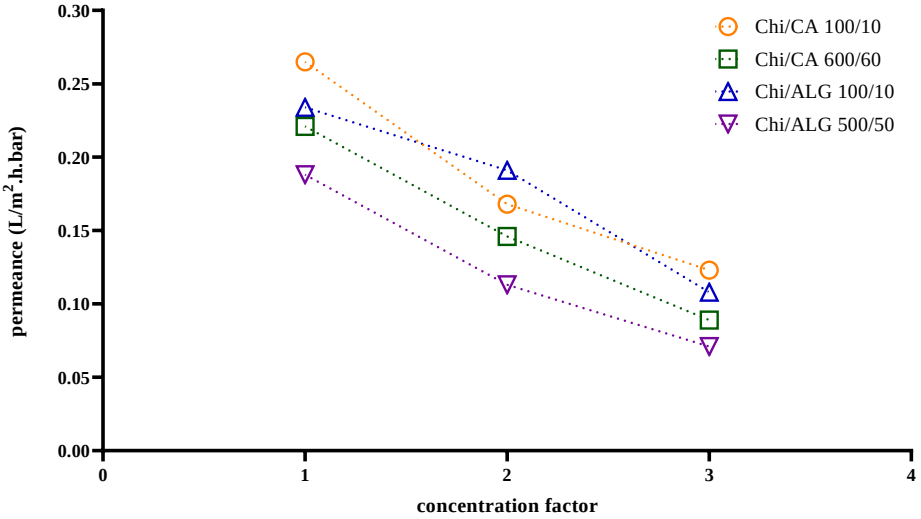


Figure 2.10. Membrane permeance as a function of volumetric concentration factor for different coagulant/flocculant combinations. Chi – Chitosan; CA- Carrageenan; ALG – Alginate.

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 [91], [92]. The highest permeance was recorded for the chitosan/carrageenan combination at the lowest concentration (0.123–0.265 L/m² h), and the lowest for the chitosan/alginate combination with the highest concentration (0.071–0.188 L/m² 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 [93].

The protein and lipid content in all combinations were also studied (**Figure 2.11**.). 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.

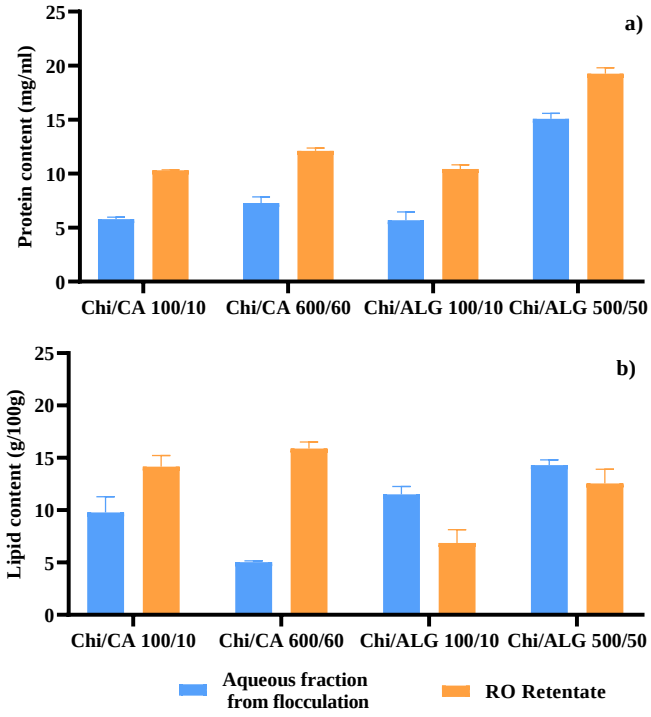


Figure 2.11. Protein (**a**) and lipid (**b**) content of reverse osmosis retentates, for different combinations of chitosan/carrageenan and chitosan/ alginate.

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 2.12**). 1-Penten-3-ol is still the main compound present in all combinations.

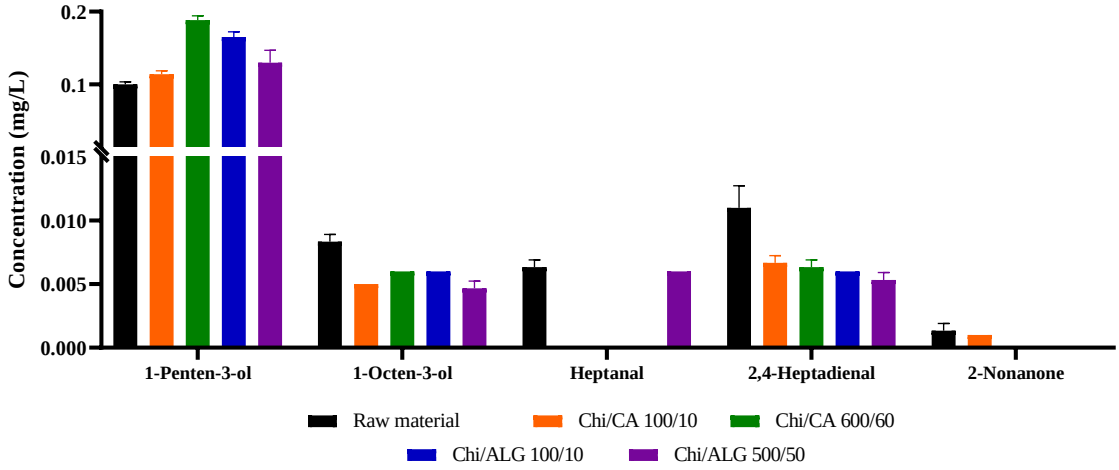


Figure 2.12. Aroma characterization of the RO retentates for different coagulation/flocculation combinations.

Focusing on the obtained permeates, in **Figure 2.13**. are presented with the values of COD obtained, to evaluate the possibility of reusing the permeate as process water.

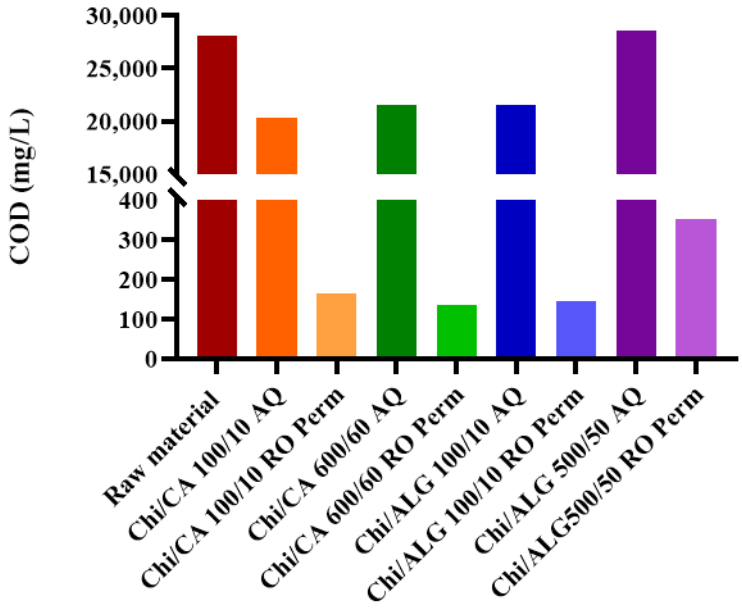


Figure 2.13. Effect of integrated system of coagulation/flocculation followed by reverse osmosis on the COD of the permeates.

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, since 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.

2.1.5. 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.

2.2. Pervaporation recovery of valuable aromas from by-products of the seafood industry: Modelling of fractionated condensation for off-flavour removal

Published as: M. João Pereira, Carla Brazinha, João G. Crespo, "Pervaporation recovery of valuable aromas from by-products of the seafood industry: Modelling of fractionated condensation for off-flavour removal", Separation and Purification Technology (2022), Vol. 286, 120441.

The author was involved in planning and performing the experiments, as well as in the discussion and interpretation of the results and preparation of the manuscript.

2.2.1. Summary

Following a circular economy approach, an integrated process is proposed for aroma recovery and fractionation from seafood industry aqueous effluents, combining the advantages of organophilic pervaporation and fractionated condensation.

The aim of this work is to recover valuable aromas from aqueous effluents of seafood processing assuring that the aromas recovered are, as much as possible, free from off-flavours to be applied as seafood flavouring additives. To reach this objective, an integrated organophilic pervaporation / fractionated condensation process was assessed, with the support of a mathematical model.

The mathematical model described and validated in this work allows for simulating the integrated pervaporation - fractionated condensation process and selecting the optimal operating conditions for a given target separation, performing a minimum experimental work (that comprises only pervaporation with total condensation experiments and inert gas molar flow rate measurements) and using reliable thermodynamic properties (saturation vapour pressures and activity coefficients).

The application of this model proves to be a very useful tool for predicting the fractionation of aromas of different chemical families. Which allows to anticipate its wider application, beyond the seafood aroma case-study discussed in this work.

2.2.2. Introduction

Food processing companies still produce considerable amounts of effluents/by-products, which are not valorised. But, in today's world, the need for more sustainable processes within a circular economy concept, is receiving growing attention. Food by-products have been studied as low-cost sources of raw materials for the generation of value-added products and energy production within a biorefinery concept [94], [95]. In the seafood industry, aqueous liquid and gaseous effluents/by-products produced during boiling processes are complex

streams that comprise aroma compounds of different chemical species, which might also include a significant amount and diversity of off-flavour compounds. The recovery of a valuable natural aroma fraction, free from off-flavours, allows these industries to counterbalance the cost of wastewater treatment through the recovery of valuable products, aromas/flavours that may be used as food additives [48], [96]. The demand for natural additives (free of off-flavours) is growing in the food/feed market and the seafood cooking effluents are regarded as a sustainable, useful feedstock for the production of natural seafood flavouring additives [97].

Organophilic pervaporation has a high potential for the recovery of natural aroma compounds, highly diluted in complex aqueous media, such as aqueous effluents, as an alternative to energy-intensive technologies such as distillation/evaporation [30], [98], since pervaporation, only a small fraction of the feed is transported through the membrane to the vacuum downstream compartment, where it is condensed. Pervaporation avoids the addition of chemical solvents and involves a lower energy expenditure than distillation/evaporation processes, due to its high selectivity towards target volatile compounds and ability to operate under mild temperatures. Additionally, under these mild conditions, it minimises the risk of degradation of aroma compounds [6], [48]. As a result, studies aimed at recovering the aroma compounds found in wastewaters present a significant scientific and technical potential. Few studies have focused on the recovery of aromas from seafood aqueous effluents by pervaporation and concluded the high potential of pervaporation as a recovery and concentration method of seafood aromas, from dilute models and real effluents[48], [55], [99], [100]. Once recovered, these aromas can be applied to improve the aroma of processed seafood products, which can be labelled as "natural flavours", since they were acquired from the original natural aqueous effluents source through a physical process, without using extracting solvent agents [55].

A combination of pervaporation and distillation is the most common hybrid approach for the fractionation of volatile compounds, particularly for the recovery of valuable natural aroma fractions free from off-flavours. Hybrid separation methods are becoming extremely important as they seem to be more appropriate for the fractionation of complex mixtures [38], [101].

The design of hybrid separation processes usually requires the need for extensive experimental studies and access to thermodynamic properties, to support the development of sound and reliable design models. However, the number of experiments needed to achieve a reasonable quantitative model may be reduced without decreasing its efficiency [38], [102], if the governing principles are correctly established.

In previous studies, Baudot & Marin [17] suggested an approach that allows for estimating the recovery of highly diluted aroma compounds from aqueous feed solutions by pervaporation, using a two-stage fractionated condensation process. The case-study described in that work was the aroma recovery from wine fermentation media, using model solutions

mimicking wine fermentation. The model developed allowed the characterisation of the permeate streams after condensation (condensates) under defined upstream pervaporation conditions and permeate downstream pressure, for a variable temperature in the first condenser. This model assumes that thermodynamic liquid-vapour equilibrium is reached in each condenser. Based on that work, and considering an identical case-study, Brazinha et. al. [10] developed a model to recover desirable aromas present in wine, which enabled to predict of the impact of the co-solvent ethanol and dissolved non-condensable gases (namely CO_2) on the recovery of aroma condensates from aqueous and hydroethanolic solutions.

Using this study as support, a modelling framework is presented to assist in the fractionation of aromas recovered from a seafood aqueous effluent, aiming at the removal of off-flavours for the production of seafood flavouring additives. This model requires as inputs: 1- the relevant operating conditions: feed composition, permeate pressure and temperature in the first condenser; 2- thermodynamic parameters: aromas saturation vapour pressure and activity coefficient and; 3- minimum experimental information: pervaporation fluxes in total condensation experiments. As it will be discussed, this model allows for predicting the mass and composition of the various aromas recovered in each condenser used. It is important to stress that, considering the difficulty of the task – the separation of valuable, desirable aromas, from off-flavours –, the separation process designed tries to benefit from the intrinsic selectivity of the membrane used and the selectivity that a fractionated condensation might offer. Therefore, two condensers in-series are used in the downstream circuit, after the pervaporation membrane module, under reduced pressure.

This study represents a step forward, extending and validating a mathematical model that applies pervaporation combined with fractioned condensation, aiming for the recovery of seafood aromas free from off-flavours. Additionally, a sensitivity analysis was performed on three key parameters required to describe the process accurately: the saturation vapour pressure, given by Antoine coefficients, the activity coefficient of each aroma compound, obtained from reported values in literature, and the inert gas flow rate, which is specific from each process/pervaporation installation, experimentally measured.

The approach described in this work may be adopted (and adapted) to other organophilic pervaporation processes that require the integration of pervaporation with fractionated condensation.

2.2.3. Experimental

2.2.3.1. Materials

2.2.3.1.1. Pervaporation membrane

The membrane used in the pervaporation studies is an organophilic dense membrane Pervap™ 4060 (DeltaMem AG, Switzerland), a commercially available flat sheet membrane,

which active layer is polydimethylsiloxane (PDMS) with a thickness of 5 μm and an effective membrane area of 0.01 m^2. The maximum long-term operating temperature is 80° C. This membrane was selected due to its good performance for the permeation of organic compounds by pervaporation [103] and its reported affinity toward seafood aromas [104].

2.2.3.1.2. Aroma compounds and the definition of the model solution of seafood aromas

A complex model solution of seafood aromas with representative aroma compounds was defined in this work. Specifically, the model solution intends to mimic an aqueous condensate obtained during the cooking of seafood, where the aromas are exposed to high temperatures for a short time. The water used in the model solution was plain deionised water, produced with a system of mixed cationic-anionic bed resins (conductivity of 0.067 μs/cm). The selected aroma compounds belong to different and relevant families of chemicals present in the real stream and exhibit different organoleptic properties, including some off-flavours formed during industrial processing. **Table 2.6. A** shows the characteristics of each selected aroma compound present in the model aqueous solution of the seafood. **Table 2.6.B** summarises the thermodynamic properties and the organoleptic characteristics of the selected aroma compounds, including activity coefficients at infinite dilution and saturation vapour pressure of these compounds. The Odour Activity Value (OAV) is defined as the ratio between the individual substance concentration in a sample and this substance's threshold concentration (the minimum concentration that can be detected by the human nose).

This work was performed at a feed temperature of 60° C, which is a relatively high-temperature value for enhancing aroma fluxes without affecting their organoleptic properties, but lower than the maximum operating temperature of the membrane Pervap™ 4060.

Table 2.6. Characteristics of the model solution of seafood and its aroma compounds: **A**, general characteristics of the aroma compounds of the model solution and **B** physicochemical properties of the aroma compounds (MW= molecular mass, BP=boiling point, pvi=vapour pressure, $\Upsilon\infty$= activity coefficient at infinite dilution, Hi= Henry's constant, OAV= odour activity value)

(A)

Aroma compound	Family of chemicals	Reason to be included in the model solution of seafood	Concentration in the model solution
1-Penten-3-ol (Sigma–Aldrich, Germany, ≥ 98% purity)	Alcohol	The responsible flavour of fresh marine products generated from polyunsaturated fatty acid [79]	4 ppm
cis-2-Penten-1-ol (Sigma–Aldrich, Germany, ≥ 96% purity)		Presented in fresh sardines and broiled mackerel [79], [81]	1 ppm
1-Octen-3-ol (Sigma–Aldrich, Germany, ≥ 98% purity)		One of the aroma compounds widely distributed in fresh and saltwater fish [82]	0.5 ppm

2,3-Pentanedione (Sigma–Aldrich, Germany, ≥ 97% purity)	Ketone	Contribute to the sweet floral, fruity flavour of many crustaceans [105]	1 ppm
2-Nonanone (Sigma–Aldrich, Germany, ≥ 99.5% purity)		Presented in broth from crab and oyster [105]	2 ppm
Heptanal (Sigma–Aldrich, Germany, ≥ 95% purity)	Aldehyde	Off-flavours that result from the thermal degradation of polyunsaturated fatty acids. The cooking method promotes the creation of various aroma compounds through the thermal oxidation and decomposition of fatty acids, resulting in the distinctive aroma of cooked fish [81]	0.6 ppm
trans, trans-2,4-Heptandienal (Sigma–Aldrich, Germany, ≥ 88% purity)			1 ppm
trans-2, *cis*-6-Nonadienal (Sigma–Aldrich, Germany, ≥ 95% purity)			0.7 ppm
Dimethyl disulphide (DMDS) (Sigma–Aldrich, Germany, ≥ 99% purity)	Sulphur compounds	Off-flavours are found in most thermally processed seafood. Due to their low threshold values, these compounds gave strong, sulphurous, cooked cabbage odours and indicate problems in sanitary quality [50], [105]	0.5 ppm
Dimethyl trisulphide (DMTS) (Sigma–Aldrich, Germany, ≥ 98% purity)			4 ppm

(B)

Compound	MW (g/mol)	$P^{vi}_{25°C}$ (Pa)	$P^{vi}_{60°C}$ (Pa)	$\Upsilon^{\infty}_{25°C}$	$Hi_{25°C}$ x10^{-4} (Pa)	OAV in the model solution
1-Penten-3-ol	86.13	1757	11529	54*	9.49	10
(*Z*)-2-Penten-1-ol	86.13	1042	8449	97	10.11	3
1-Octen-3-ol	128.22	104	832	3568	37.11	500
2,3-Pentanedione	100.12	596	4503	207*	12.34	50
2-Nonanone	142.24	77	658	16200	124.74	400
Heptanal	114.19	443	3237	4180	185.17	200
(*E*,*E*)-2,4-Heptadienal	110.16	38248	402398	2960	11321.41	10000
(*E*2, *Z*6)-Nonadienal	138.21	7460	71590	23742	17711.53	70000
Dimethyl disulphide	94.19	62900	208065	183*	1151.07	167
Dimethyl trisulphide	126.26	62012	205563	17	105.42	400000

* Activity coefficient at infinite dilution for 50 °C. No values were found for the temperature of 60 °C. MW- molecular weight; t; p_{vi} – saturation vapour pressure; *Hi* – Henry's constant; OAV – odour activity value**.**

2.2.3.2. Experimental set-up

2.2.3.2.1. Pervaporation experimental set-up

Experimental setup A using a single total condenser, shown in **Figure 2.14.A**, was designed to recover aromas by pervaporation, to obtain information about the total individual flux of each feed stream component. This setup consists of a pervaporation cell with the feed

stream circulating at a controlled flow rate and feed temperature. The downstream circuit is constituted of one total condenser working at a controlled permeate pressure. The module used was a radial flow flat module (GKSS, Germany) which is described in detail in [34]. The feed enters the module centrally, flows radially along the membrane, and exits at the circular periphery of the membrane. The upstream tubing was either of Viton or Teflon and the metal used in the unit was stainless steel. The vacuum conditions on the permeate side were assured by an E2M5 rotary vane dual-stage mechanical vacuum pump (Edwards, UK). The unit was equipped with a TPR280 pressure gauge (Pfeiffer Vacuum, Germany), with readings of the permeate pressure, p_{perm} [Pa], independent from the nature of the gas or vapour present. An RVC 300 controller (Pfeiffer Vacuum, Germany) controlled the downstream pressure, pperm [Pa], by varying the resistance created by an electro valve (V2) RME 005 (Pfeiffer Vacuum, Germany). The total condensation occurs using a U-shape trap immersed in liquid nitrogen.

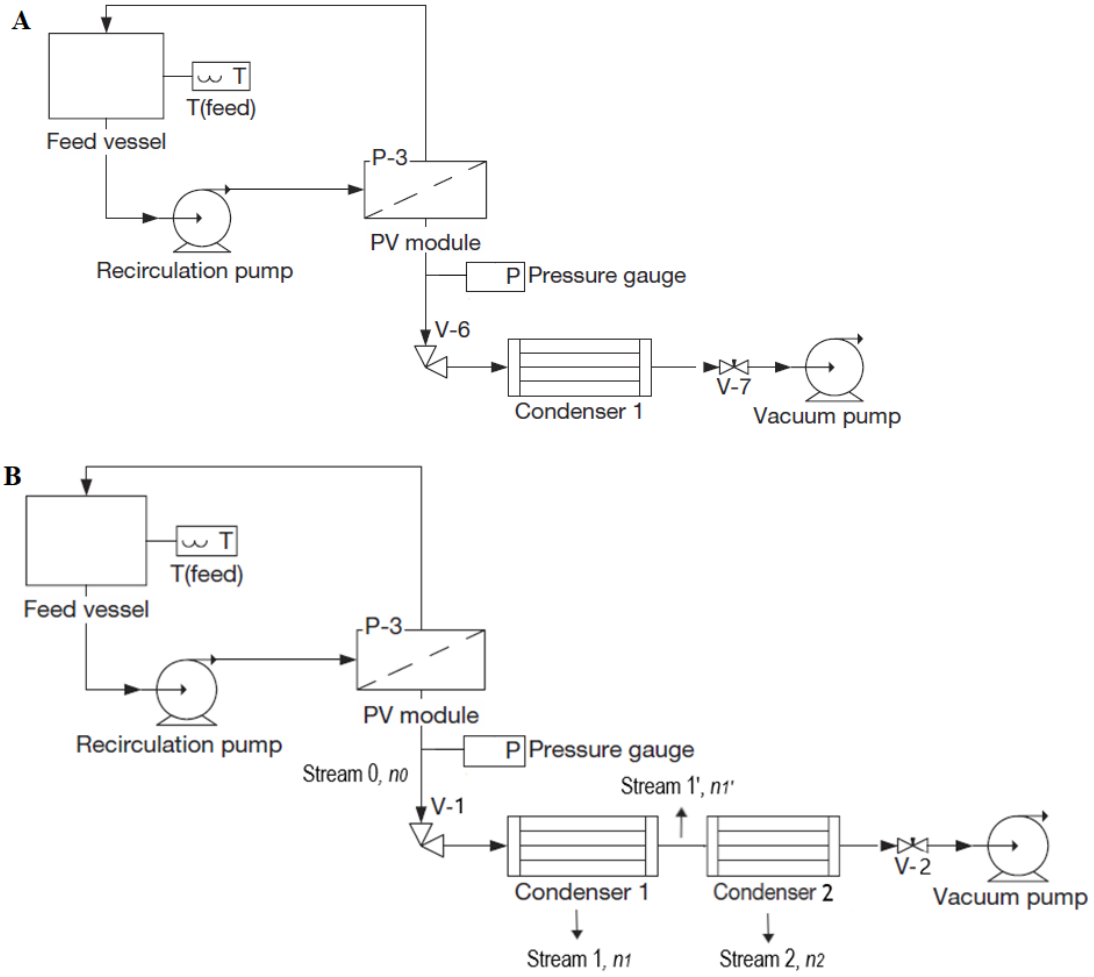


Figure 2.14. Schematic representation of the experimental setup of a pervaporation lab unit with total condensation (A) and equipped with two vacuum condensers-in-series (B).

2.2.3.2.2. Pervaporation-fractionated condensation experimental setup

The experimental setup B, shown in **Figure 2.14.B**, was designed to pervaporate and fractionate the aromas, respectively by pervaporation and by fractionated condensation. The upstream circuit was the same as described in section 2.2.3.2.1. However, in this case, the downstream circuit integrates two in-series condensation U-shape glass trap condensers. The first condenser was immersed in a refrigerated bath with adjustable temperature from -25 to 200 °C, using a Corio CP-300F (Julabo, Germany). The second condenser was immersed in liquid nitrogen. The permeate circuits of the pervaporation experimental setup (**Figure 2.14.A**) and the pervaporation-fractionated condensation experimental setup (**Figure 2.14.B**) do not include restriction valves or an additional vacuum pump between the two condensers. Since vacuum pumps in lab setups are typically over-dimensioned, the permeate pressure can be considered constant in the downstream circuit, defined by the vacuum pump and the RVC 300 controller (Pfeiffer Vacuum, Germany).

2.2.3.3. Operating conditions

2.2.3.3.1. Feed compartment

The feed was a model aqueous solution containing the selected seafood aromas with the concentrations listed in **Table 2.6.** Characteristics of the model solution of seafood and its aroma compounds: **A**, general characteristics of the aroma compounds of the model solution and **B** physicochemical properties of the aroma compounds (MW= molecular mass, BP=boiling point, pvi=vapour pressure, $\Upsilon\infty$= activity coefficient at infinite dilution, Hi= Henry's constant, OAV= odour activity value). To minimise the depletion of aromas in the feed solution during each experiment, the volume of the feed was sufficiently large (10 L) to avoid significant changes in concentration, and the feed vessel was kept closed with a reduced headspace volume. Additionally, considering the volume of the feed solution *versus* the membrane area of 0.01 m^2, the duration of each experiment was set to 1 hour. Despite the reduced experimental time, careful membrane conditioning was previously performed to guarantee that the membrane was equilibrated in terms of water content, by pervaporating water for 5 hours, before each pervaporation experiment.

2.2.3.3.2. Pervaporation experiments with total condensation

During the pervaporation experiments, the following parameters were controlled: temperature of the feed stream T_{feed} at 60 ± 1 °C, average Reynolds number in the feed compartment Re_{feed} [-] of 430 [10] and temperature of the condenser at -196 °C (with liquid nitrogen), for assuring a total vapour condensation. The permeate pressure, p_{perm} [Pa] was adjusted to 200, 1000 and 1500 Pa in distinct experiments.

2.2.3.3.3. Pervaporation-fractionated condensation experiments

In the pervaporation experiments followed by fractionated condensation (pervaporation-fractionated experiments), the following parameters were controlled: temperature of the feed stream T_{feed} at 60 ± 1 °C and average Reynolds number in the feed compartment Re_{feed} [-] of 430. The temperature of the first condenser $T_{1,condens}$ was set at -15.0, -7.5 and 0.0 °C according to the corresponding study and the temperature of the second condenser $T_{2,condens}$ was at -196 °C.

In this work, the selected permeate pressure p_{perm} was 1000.0±50.0 Pa, which implies a moderate energy expenditure, assuring simultaneously an adequate permeate flux, as discussed in Brazinha *et. al.* [10].

2.2.3.4. Analytical methods

Gas chromatography analyses were performed to quantify the aroma compounds in the different condensates obtained. A Shimadzu gas chromatograph (GCMS-QP2010) equipped with a WAX column (30 m x 0.25 mm i.d. x 0.25 μm) was used during the study. Ultrapure helium at 1 mL/min was used as the carrier gas. The oven temperature program was: 60° C (held for 4 min), 2° C/min to 180° C. The injector temperature was set at 200° C, limited by the temperature of desorption indicated by the SPME fibre manufacturer. Detector temperature was set at 220° C, ionisation source at 200° C and the ionisation mode was electron impact with an electron energy of 70 eV. A volume of 6 mL was extracted with a CAR/PDMS fibre for 15 min at 60° C. The time for analyte desorption from the SPME fibre was fixed at 10 min. The injection was performed in the splitless mode for 2 min. After this time, the split ratio was set at 1:20 until the end of the chromatographic run. 2-nonanol was used as an internal standard for the analysis of the samples.

2.2.4. Theory

2.2.4.1. Pervaporation

In a pervaporation process, the equation of mass transport allows to relate the driving force for transport of compound *i* through the membrane (the difference of its partial pressures in the feed and permeate side contacting the membrane) and its partial flux with the overall mass transfer coefficient, $k_{ov,i}$ [mol.m^{-2}. s^{-1}. Pa^{-1}], as in eq. 8 and 8':

$$Ji = K_{ov,i} \cdot \left(p_{i,feed} - p_{i,perm}\right) \quad (8)$$

$$Ji = K_{ov,i} \cdot \left(x_{i,feed} \cdot \Upsilon_{i,feed} \cdot p_{vi\,(Tfeed)} - y_{i0} \cdot p_{perm}\right) \quad (8')$$

where J_i [mol m^{-2} s^{-1}] is the transmembrane flux of permeating compound *i*. The partial pressure of compound *i* in the feed compartment $p_{i,\,feed}$ [Pa] and the partial pressure of compound *i* in the permeate compartment $p_{i,\,perm}$ [Pa] are respectively calculated by the modified Raoult's and Dalton's laws (see eq. 8'). The parameters related to the feed solution are the

molar fraction of i, $x_{i,\,feed}$ [-]; the activity coefficient of i, $\gamma_{i,\,feed}$ [-], and the saturation vapour pressure of i p_{vi} [Pa]. At the permeate compartment, the partial pressure of compound i in the permeate stream before any condenser may be represented by $y_{i0} \cdot p_{perm}$ (see eq. (8'), where y_{io} is the molar fraction of i in the permeate and p_{perm} is the total pressure in the permeate.

The membrane permeability (Li) is given by Eq. (9)

$$J_i = K_{ov,i} \cdot driving\ force \leftrightharpoons J_i = \frac{L_i}{\delta} \cdot \left(x_i \cdot \gamma_i \cdot p_{vi(T)} - y_i \cdot p_{perm}\right) \quad (9)$$

where Li is the membrane permeability, which is a product of diffusivity and solubility coefficients, δ is the membrane thickness, x_i [-] and y_i [-] are the feed and permeate molar fraction, respectively, and p_{perm} [Pa] is the permeate pressure.

It is assumed that the mass transfer resistance is associated with transport across the membrane and no effects of concentration polarisation are considered. Therefore, values of permeability were able to be calculated, using Eq. (9). The permeability and selectivity are important parameters to quantify the performance of pervaporation processes. The selectivity of compound i in relation to compound j is calculated by eq. 10:

$$Selectivity = \frac{L_i}{L_j} \quad (10)$$

where $L_i\,[m]$ and $L_j\,[m]$ are the permeability of compounds i and j, respectively.

The separation factor is defined according to Eq. 11.

$$Separation\ factor\ (\alpha_{i/j}) = \frac{(C_i^{Perm}/C_i^{Feed})}{(C_j^{Perm}/C_i^{Feed})} \quad (11)$$

2.2.4.2. Model of pervaporation-fractionated condensation

The mathematical model was developed considering a fractionated condensation with two condensers in-series, as shown in **Figure 2.14.B**. This model aims to predict the recovery of aromas at a given permeate pressure, supported by an efficient and optimised fractionated condensation. To obtain target condensate compositions, the model was used to define optimal operating temperatures in the first condenser (at a fixed permeate pressure of 1000 Pa) for the separation of compounds with desirable organoleptic properties (in this case, alcohols and ketones) from other compounds present, namely water and off-flavours (sulphur compounds and aldehydes).

As shown in the diagram in **Figure 2.15**, the parameters related to the feed side of pervaporation were kept constants for all model simulations: 1) feed conditions (composition, temperature and membrane) and 2) thermodynamic properties of compound i present in the system (saturation vapour pressure p_{vi} [Pa] and activity coefficient γi [-], at the feed temperature in an aqueous media). Additional information was considered: the inert gas molar flow rate, total permeate pressure and, consequently, the permeate flux of each aroma under study.

Due to the lack of published data concerning the saturation vapour pressures and activity coefficients at 60 °C, values at 25 and 50° C were considered for the activity coefficients. To access the impact of the thermodynamic parameters used for modelling, a sensitivity analysis was performed to infer the impact of saturation vapour pressure, activity coefficient and the inert gas molar flow rate. This analysis is presented as Supplementary Material.

The temperature of the first condenser, $T_{1,condens}$ [°C], is the single operating parameter which is left as a free operating parameter because it is the parameter that will determine the composition of the condensates in the 1st and the 2nd condenser when the other parameters (feed conditions and permeate total pressure) are defined.

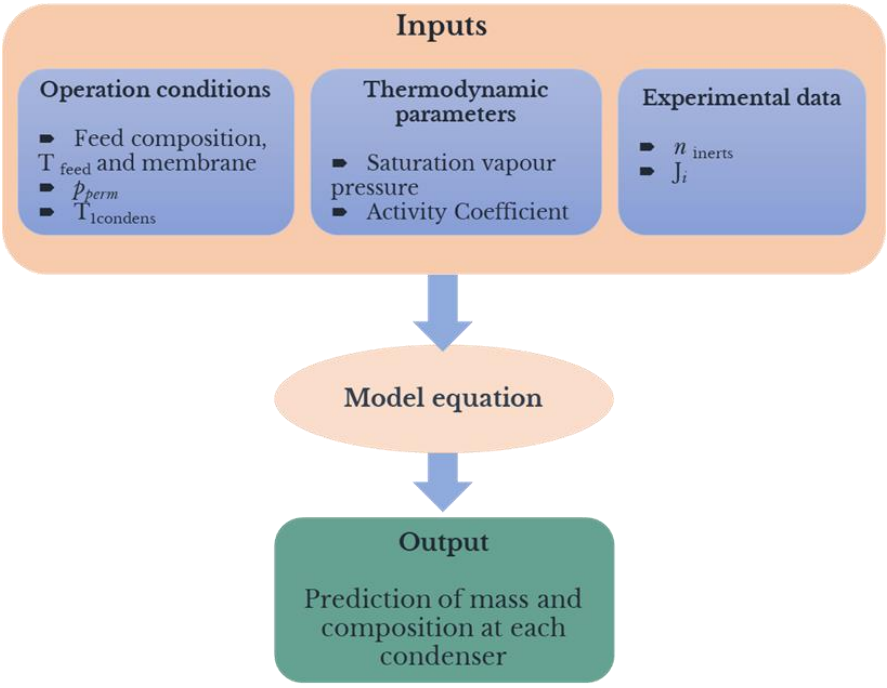


Figure 2.15. Diagramme of input variables and outputs of the model applied in this work.

For the application of this model, a number of assumptions were considered, according to the chosen operating conditions, namely: 1) the system works in a steady-state (which is reasonable, considering the large volume of feed used); 2) the inert gas molar flow rate is constant along time and the compounds present are not condensed (flow of non-condensable gases); 3) the thermodynamic equilibrium is maintained in each condenser of the in-series system (acceptable, when sufficient contact time is allowed); 4) the activity coefficients are independent of temperature in the range of temperature values under study as referred in [106] (this assumption is discussed later and the impact of the assumed values of activity coefficients is assessed through a sensitivity analysis), and 5) the transport of vapours in the permeate compartment do not experience a resistance to diffusion (assured by the vacuum applied).

The model described in this work, with the following equations and the streams identified in **Figure 2.14.B**, addresses systems where the feed solution is an aqueous solution containing dilute compounds. From the flux of each compound *i* present in the system, which is obtained experimentally, and its thermodynamic properties under defined feed conditions and defined permeate pressure, it is possible to estimate the mass of each compound and the composition of the two condensates. This is an extremely useful model to assist in the selection of best-operating conditions, to reach a target separation between valuable aromas and off-flavours.

$$\%condens_{iw} = \frac{n_{w1}}{n_{w0}} = 1 - \frac{n_{w1'}}{n_{w0}} \quad (12)$$

$$\frac{n_{w1'}}{n_{inert}} = \frac{p_{vw(T1,condens)}}{p_{perm} - p_{vw(T1,condens)}} \quad (13)$$

$$p_{w1'} = p_{vw(T1,condens)} \quad (14)$$

$$\%condens_{w1} = 1 - \frac{n_{inert}}{n_{w0}} \cdot \frac{p_{vw(T1,condens)}}{p_{perm} - p_{vw(T1,condens)}} \quad (15)$$

The percentage of water condensation in the first condenser is obtained by Eq. (12). The water molar flow rate after the first condenser, n_{w1}', is calculated from Eq. (13), where the ratio of n_{w1}' and inert gas molar flow rate in the stream between condensers (stream 1') is assumed to be equal to the ratio of water partial pressure and inert gas partial pressure in the same stream, implying that the vapour behaves as an ideal gas (Dalton's law).

Assuming thermodynamic equilibrium in the first condenser, Eq. (14), the water condensation percentage in the first condenser is obtained with Eq. (15).

When applying this model to real or model solutions, we are able to deal with complex matrixes with aroma compounds in highly diluted concentrations. According to Lipnizki *et. al,* [107] the aroma compounds exhibit an autonomous behaviour with no flux interaction between them. Therefore, using the same reasoning discussed above, the following series of equations might be established.

$$p_{perm} = p_{inert,1'} + p_{w,1'} + p_{aroma,1'} \cong p_{inert,1'} + p_{w,1'} \quad (16)$$

$$\frac{n_{i1'}}{n_{inert}} = \frac{p_{i1'}}{p_{inert}};\ \ i: water, aroma \quad (17)$$

$$\%condens_{aroma1} \cong 1 - \frac{n_{inert}}{n_{aroma0}} \cdot \frac{\varkappa_{aroma1} \cdot \gamma^{\infty}_{aroma1} \cdot p_{varoma(T1,condens)}}{p_{perm} - p_{vw(T1,condens)}} \quad (18)$$

Due to their vestigial concentrations, the contribution of the aroma(s) to the permeate pressure, p_{perm}, and the total permeate molar flux in a given stream can be neglected (Eq. 16)). In Eq. (17), as occurs in Eq. (13), the ratio of water molar flux and inert gas molar flow rate in the stream between the two condensers (stream 1') is considered be equal to the ratio of water partial pressure and inert gas partial pressure in the same stream. In the end, the expressions

for calculating the percentage of condensation of water and aroma(s) in the first condenser are obtained, respectively, by Eqs. (15) and (18).

2.2.4.3. Calculation of model parameters: volume of permeate, inert gas molar flow rate and the saturation vapour pressure

To calculate the inert gas molar flow rate, n_{inert} [mol/s], an estimation of the downstream circuit volume, V_{perm} [ml], is required. V_{perm} was estimated through Eq.(19), by changing this circuit's volume in a precise mode (by adding inert spheres with calibrated volumes) and measuring the corresponding downstream pressure. The V_{perm} determined was 780 ± 10 ml.

$$p_{initial} \cdot V_{perm} = p_{final} \cdot (V_{perm} + V) \Leftrightarrow \frac{p_{initial}}{p_{final}} = 1 + \frac{1}{V_{perm}} \cdot V \quad (19)$$

Due to unavoidable air leakage in the experimental set-ups (as happens in any industrial module), as a consequence of the lack of membrane sealing in the pervaporation module and of leaks at the interfaces of the downstream circuit, the inert gas molar flow rate was measured, at 60 °C. A series of pervaporation experiments were carried out with water after the system reaches the required temperature and minimum pressure value, after which the needle valve, near the vacuum pump, was closed and the increasing downstream pressure, p_{perm} [Pa], was monitored over time. The inert gas molar flow rate to the downstream circuit, n_{inert} [mol/s], can be calculated through the ideal gas equation using the slope of the function $\Delta p_{perm}/\Delta t$ [Pa/s], assuring (experimentally confirmed) that there is no driving force for water vapour transport (using permeate pressure values above the saturation vapour pressure of water at 60 °C). The value of n_{inert} on this set-up was experimentally found to be $6.74 \times 10^{-7} \pm 4.00 \times 10^{-8}$ mol.s^{-1}.

The saturation vapour pressure of water and selected aromas was calculated using the Antoine law equation referred to as the operating temperature, using the Antoine law constants listed in Table 2.7.

Table 2.7. Antoine law constants for each compound studied

Compound	A	B	C	Range of temperature (°C)	Reference
Water	4.65	1435.26	-64.85	-16.25 to 99.85	[108]
1-Penten-3-ol	7.75	1653.43	224.23	14.19 to 137.39	[109]
(*Z*)-2-Penten-1-ol	7.61	1502.48	198.69	-78.15 to 278.85	[110]
1-Octen-3-ol	6.79	1602.63	207.39	69.45 to 236.86	[109]
Heptanal	7.32	1635.48	215.53	-43.00 to 329.85	[110]
(*E,E*)-2,4-Heptadienal	5.48	686.45	111.82	-	[111]
(*E*2, *Z*6)-Nonadienal	7.82	2036.85	227.49	-	[111]
2,3-Pentanedione	7.44	1599.97	210.66	-23.20 to 382.85	[110]
2-Nonanone	4.72	2030.50	-37.95	33.15 to 195.85	[108]

Dimethyl disulphide	7.08	1150.87	236.49	-0.15 to 59.85	[109]
Dimethyl trisulphide	4.29	1201.13	-29.91	-21.55 to 21.09	[108]

2.2.5. Results and discussion

2.2.5.1. Processing of the model solution of seafood aromas at 1000 Pa by pervaporation: experimental characterisation

The pervaporation experiments of the model solution under defined upstream operating conditions produced permeate streams, which are the starting-point of the model developed in this work.

The performance of pervaporation is, in general, characterised by aroma permeabilities and selectivities. These two parameters enable the comparison of different pervaporation processes at the same temperature under different experimental operating conditions (e.g. feed compositions, permeate pressure) [112]. The pervaporation of seafood aromas is reported in the literature, particularly in the processing of oyster boiling wastewaters with T_{feed} of 50 °C and a p_{perm} of 700 Pa obtaining permeate fluxes of 0.2 x 10^{-8} g/(m^2.s) and separation factors of 4 for 1-octen-3-ol against water [55]. Martinez [48], using a brown crab effluent at T_{feed} of 40 °C obtained enrichment factors around 3 for 1-penten-3-ol and 2-penten-1-ol, 8.3 for 1-octen-3-ol, 2.5 for heptanal, 4.4 for 2,3-pentanedione, 11.9 for 2-nonanone and around 1.5 for sulphur compounds (enrichment factors against water).

Table 2.8 shows the individual fluxes [mol/(m^2.s)] and the permeabilities [mol/(m^2.s.Pa)] to the aromas under study, as well as the separation factor (calculated against water). The aromas are present in similar concentrations in the feed (10^{-1}-10^{0} ppm), but they show quite different values of J_i and L_i.

Table 2.8. Experimental parameters of pervaporation at 1000 Pa: aroma flowrate (Ji), permeability (Li) and, separation factor and selectivity of DMTS related to desirable aromas

Compound	J_i [mol/m^2.s]	L_i [mol/(m.s.Pa)]	Separation factor [-]
Desirable aromas			
1-Penten-3-ol	$2.58x10^{-8} \pm 2.75x10^{-9}$	$8.74x10^{-14} \pm 1.14x10^{-14}$	2.55±0.17
(*Z*)-2-Penten-1-ol	**$1.27x10^{-8} \pm 6.04x10^{-10}$**	**$1.10x10^{-13} \pm 2.51x10^{-14}$**	**5.28±1.07**
1-Octen-3-ol	$9.24x10^{-9} \pm 1.12x10^{-10}$	$4.33x10^{-14} \pm 3.52x10^{-15}$	5.64±0.41
2,3-Pentanedione	$3.36x10^{-8} \pm 4.87x10^{-10}$	$4.48x10^{-14} \pm 9.37x10^{-15}$	8.72±0.78
2-Nonanone	$5.96x10^{-8} \pm 4.86x10^{-10}$	$2.36x10^{-14} \pm 1.20x10^{-15}$	14.03±0.33
Off-flavours			
Heptanal	$1.26x10^{-8} \pm 4.19x10^{-10}$	$1.24x10^{-14} \pm 1.05x10^{-15}$	5.25±0.21
(*E*,*E*)-2,4-Heptadienal	$1.35x10^{-8} \pm 2.36x10^{-10}$	$1.39x10^{-16} \pm 2.09x10^{-17}$	4.55±0.21
(*E*2, *Z*6)-Nonadienal	$1.44x10^{-8} \pm 9.63x10^{-10}$	$1.77x10^{-16} \pm 5.59x10^{-18}$	7.92±0.59

Dimethyl disulfide DMDS	$2.45x10^{-8}\pm 1.30x10^{-9}$	$3.77x10^{-15}\pm 6.60x10^{-16}$	8.66±0.97
Dimethyl trisulfide DMTS	$\mathbf{2.57x10^{-7}\pm 7.78x10^{-9}}$	$\mathbf{2.55x10^{-13}\pm 1.10x10^{-14}}$	**26.62±0.84**

Although the PERVAP 4060 membrane is the most referred membrane for the recovery of seafood aromas [107], the values of permeability to the different aromas present were generally moderate.

The important and abundant off-flavour DMTS with the highest values of J_i and L_i was more efficiently removed from the feed solution than any other aroma present. This result might be expected since sulphur compounds are more organophilic, due to the sulphur atom being less polar than oxygen [113] and, hence, higher permeabilities could be expected. Still, the other sulphur off-flavour, DMDS, shows values of L_i two orders of magnitude lower than DMTS.

The desirable aromas of alcohols and ketones, with similar permeation behaviour, show values L_i one order of magnitude lower than DMTS. The exception is (*Z*)-2-Penten-1-ol with a value of L_i in the same order of magnitude as DMTS.

2.2.5.2. Pervaporation-fractionated condensation processing of the model solution of seafood aromas at 1000 Pa

To accomplish an effective removal of off-flavours from desirable aromas, the fractionated condensation step aims at obtaining two condensates, one of them enriched in desirable aromas and free from off-flavours (if thermodynamically possible). The model developed in this work was applied at a permeate pressure of 1000 Pa, where the percentage of compound *i* that is condensed/recovered in the first condenser, $\%Condens_{i1}$, was predicted for different values of $T_{1,condens}$ [° C]. Figure 2.16. shows the experimental and predicted values of the percentage of each compound *i* that is condensed in the first condenser, $\%Condens_{i1}$, against the $T_{1,condens}$.

The best possible scenarios at 1000 Pa would be to apply a condensation temperature between 0 and 5° C, where the 3 most important off-flavours (DMTS, DMDS and heptanal) have low values of $\%Condens_{i1}$, unlike most desirable aromas (namely, the alcohols and the ketone 2,3-pentanodione). Therefore, ideally, at a defined $T_{1,condens}$ value, the desirable aromas would be condensed in the first condenser and the off-flavours in the second condenser (at a lower temperature).

At a $T_{1,condens}$ [°C] of 0 °C, the model estimates an absence from the 1st condenser of almost all off-flavour DMDS ($\%Condens_{i1}$ of ~ 3 %) and 80 % of DMTS ($\%Condens_{i1}$ of ~ 20 %), as aimed. However, for the other off-flavours, it estimates that 30 % of heptanal and 2,6-nonadienal are still captured in the 1st condenser under such conditions, as well as 55 % of 2,4-heptadienal.

Nevertheless, the aldehydes 2,6-nonadienal and 2,4-heptadienal are present in trace concentrations. Regarding the desirable aromas, alcohols and the ketone 2,3-pentanedione, are estimated to be recovered, respectively, at ~70 and 90 %. However, only 40 % of the ketone 2-nonanone is estimated to be recovered.

At a $T_{1,condens}$ [° C] of 5 °C, the model estimates, for the 1st condenser, a very low capture of 10 % (or lower) of all sulphur compounds (DMDS and DMTS) and the aldehydes (%$Condens_{i1}$ of ~ 10 %), except for 2,4-heptadienal which is estimated to be condensed at 24 %. Despite these improvements in the off-flavours removal from the desirable aromas (low concentration of off-flavours in the 1st condenser), applying this temperature would represent a loss of desirable compounds since only 40 to 65 % of the alcohol amount present and only 15 % of the 2-nonanone amount are estimated to be recovered in the 1st condenser.

2.2.5.2.1. Model validation with experimental values

The values of %$Condens_{i1}$ related to this system were simulated based on Section 2.2.4.2. using as input parameters: $\varkappa_{i,\,feed}$, T_{feed}, p_{perm}, J_i, and n_{inert}, and varying $T_{1,condens}$. The feed stream composition, the model solution of seafood aromas, was presented in Section 2.2.3.1.2. (See Table 2.6.A). Figure 2.16 reveals a high agreement between the experimental and the simulated results of %$Condens_{i1}$ and, consequently, the composition of condensates at various temperatures of the first condenser. This model provides a simple and efficient method for estimating the percentage of condensation of each compound in each condenser at a given downstream pressure and condenser temperature and the resulting condensate composition. It allows to compare and define fractionated condensation strategies, according to the aim of the industrial process. A total aroma concentration strategy might be set, where all the aromas are recovered and concentrated in the first condenser, removing the most solvent from the second condenser. An aroma fractionation strategy is discussed in this work, aiming at the recovery of specific desirable aromas in the first condenser, separated from other aromas, off-flavours, captured in the second condenser. By using this approach it is possible to anticipate the best separation between desirable and undesirable compounds and define the best strategy to achieve a target separation. However, in the particular case that is under discussion, the total separation off-flavours cannot be achieved.

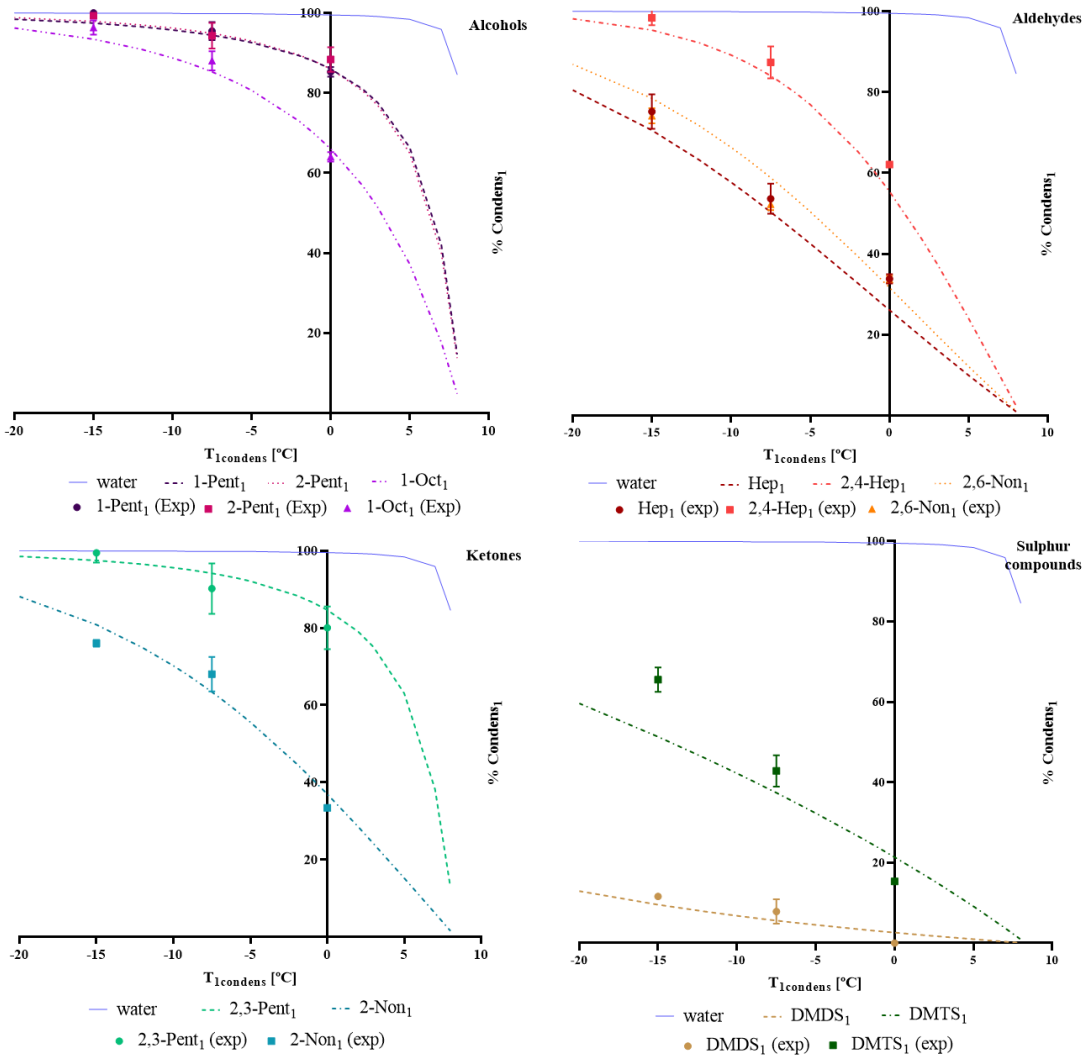


Figure 2.16. Model simulation and experimental validation for four different chemical families (Alcohols, Aldehydes, Ketones and Sulphur compounds) included in the model solution. Percentage of condensation of each compound present (water, 1-penten-3-ol, 2-penten-1-ol, 1-octen-3-ol, heptanal, 2,4-heptadienal, 2,6-nonadienal, 2,3-pentanedione, 2-nonanone, dimethyl disulphide and dimethyl trisulphide) in the 1st condenser (%Condensi1). Operating conditions: Pervap 4060 membrane; Tfeed=60° C; pperm =1000 Pa; Lines refer to simulated values and symbols to experimental data.

2.2.5.3. The effect of permeate pressure on pervaporation and pervaporation-fractionated condensation processes

The permeate pressure is a very critical parameter in pervaporation. At constant feed conditions (composition and temperature), a change in the permeate pressure has a significant effect on the permeation of each compound present in the model solution of seafood aromas by changing their driving force across the membrane (by changing their partial permeate pressure) and, consequently, their partial flux, their overall mass transfer coefficient and their permeability.

Since we could not achieve a "perfect" separation of the off-flavours from the desirable aromas by operating with a permeate pressure of 1000 Pa, other permeate pressures were also

evaluated (200 and 1500 Pa) within the range of values of permeate pressure recommended for vacuum pervaporation (below 2000 Pa) [114].

Figure 2.17 presents the effect of permeate pressure on permeate flux [mol/(m².s)], overall mass transfer coefficient [mol/(m².s.Pa)], apparent permeabilities [mol/(m².s.Pa)] of each aroma and selectivity of DMTS (off-flavour) relatively to desirable compounds, $\alpha_{dimethyl\ trisulfide\text{-}j}$ [-].

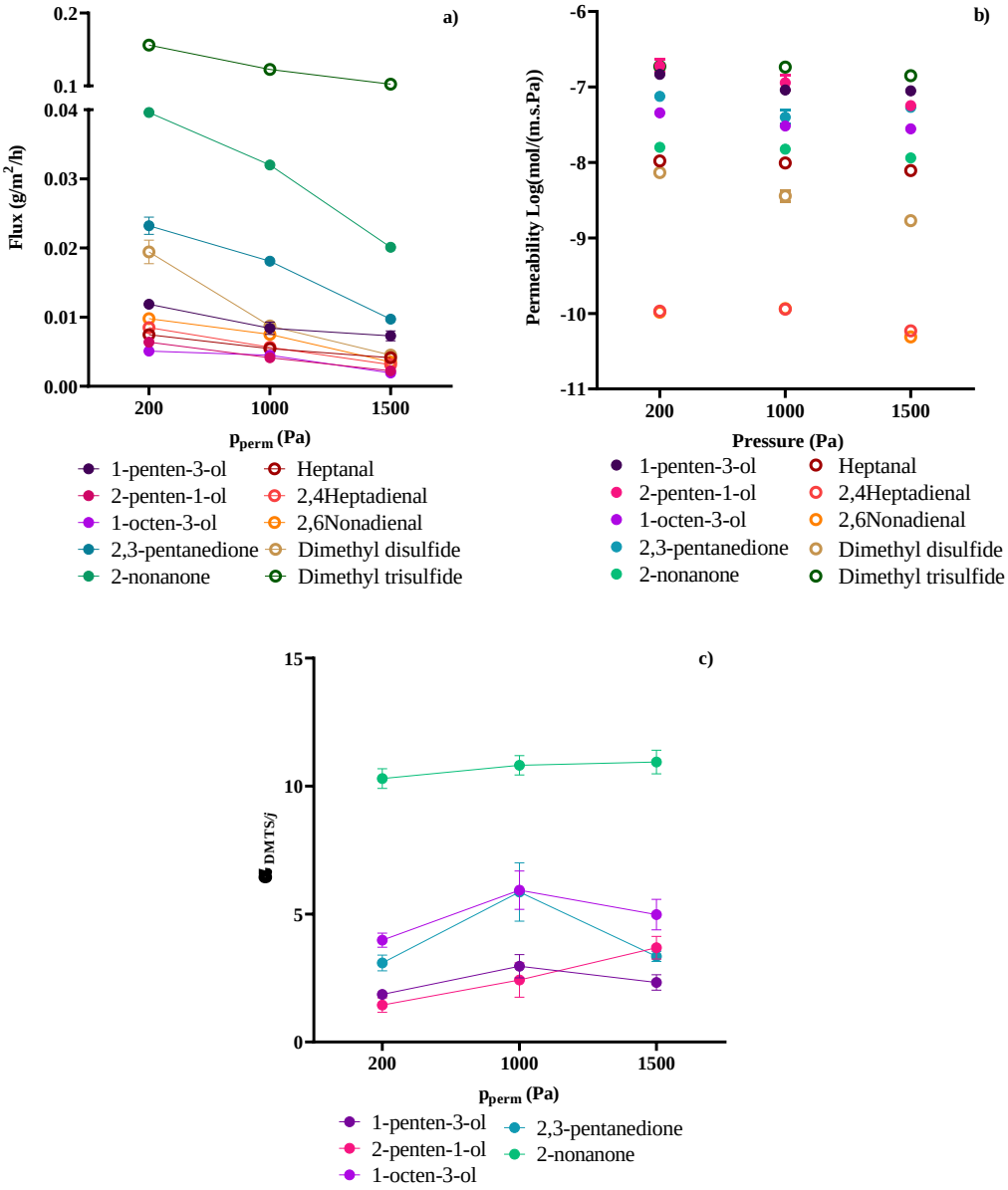


Figure 2.17. Effect of permeate pressure on a) permeate flux, b) permeabilities for each aroma and c) selectivity of DMTS relatively to desirable compounds. Each graph represents desirable aromas (filled symbols) and off-flavours (empty symbols) from different chemical families.

As expected, the overall flux of each aroma increased as the permeate pressure decreased (Figure 4a). At 60 °C feed temperature, the water molar flux decreased from 0.0179

mol $m^{-2}s^{-1}$ at 200 Pa total permeate pressure to 0.0137 mol $m^{-2}s^{-1}$ at 1500 Pa. Martinez [82] obtained similar results (water molar flux of 0.01 mol $m^{-2}s^{-1}$) using the same membrane with a feed temperature of 40 °C and a permeate pressure of 300 Pa.

These results show that pervaporation, as a stand-alone process, does not allow for a complete separation between desirable aromas and off-flavours. Therefore, the pervaporation-fractionated condensation process was studied at different permeate pressures, aiming at selecting a permeate pressure value that could significantly improve the aimed separation.

As the model developed was successfully validated experimentally at 1000 Pa, the same model of fractionated condensation was applied to the other permeate pressures selected for evaluation, 200 and 1500 Pa, to find the optimal operating conditions. The simulation results obtained are presented in Figure 2.18.

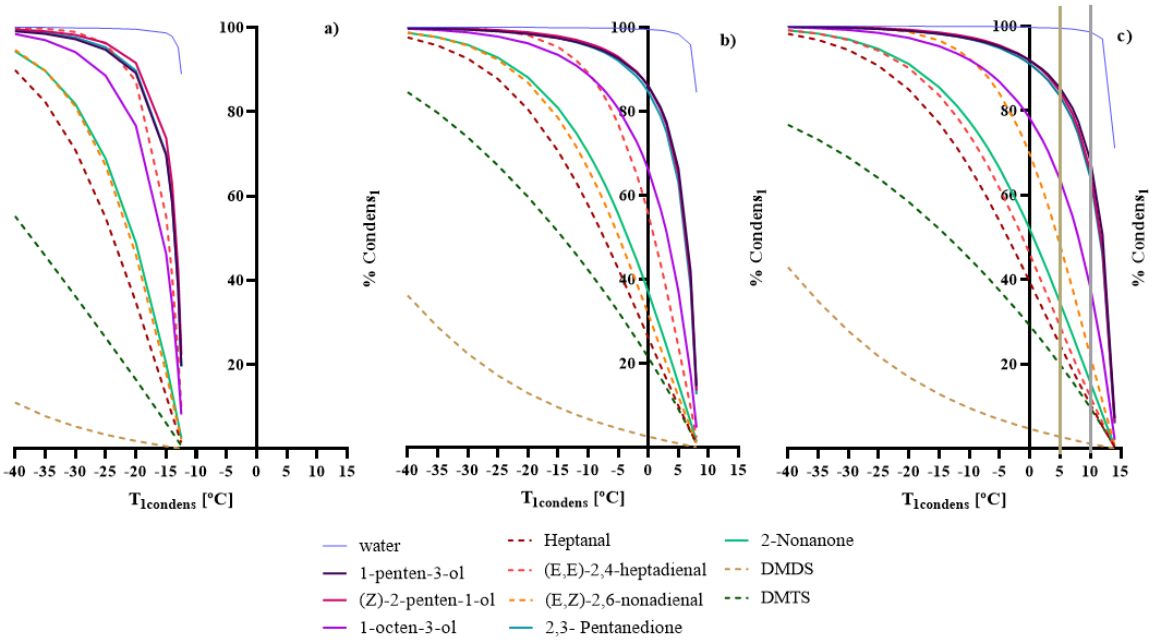


Figure 2.18. Simulation of the percentage of condensation (water and aromas) in the 1st condenser (*%Condens*$_{1}$) considering three different permeate pressures: a) 200 Pa b) 1000 Pa and c) 1500 Pa. Lines represented the simulation results for the desirable aromas and the dashed lines represents simulations for off-flavours.

The models obtained for different permeate pressures lead to different temperature ranges, as at 200 Pa the water condenses at -12 °C, while at 1000 Pa it condenses at 8 °C and 1500 Pa condenses at 14 °C. From an economic perspective, it should be stressed that operating at a downstream pressure of 200 Pa implies the use of lower pressure and condensation temperatures, representing a higher energy expenditure. Opposingly, operating with a downstream pressure of 1500 Pa is economically favourable due to less demanding vacuum pressure conditions and higher condensation temperatures.

In the modelling of fractionated condensation at a specific permeate pressure, several parameters are constant, both the upstream (saturation vapour pressure, activity coefficient

and the inert gas flow rate) and the downstream (permeate flow rate and overall mass coefficient). The only parameter that varies for each tested permeate pressure is the condensation temperature of the first condenser applied ($T_{1,condens}$ [°C]). To achieve an adequate fractionation of the off-flavours, the selected permeate pressure and temperature conditions must allow for obtaining a $\%Condens_{i1}$ of desirable aromas as different as possible from the off-flavours.

When selecting a permeate pressure of 1500 Pa and $T_{1,condens}$ [°C] of 5 °C (see a vertical line in Figure 5c), an almost total absence of the sulphur off-flavour compounds was achieved in the first condenser (with $\%Condens_{i1}$ <20 % of DMTS and <5 % of DMDS) and a $\%Condens_{i1}$ between 25-50 % for the aldehydes. Thus, the condensate obtained in the first condenser is estimated to have over 64-85 % of desirable aromas (with $\%Condens_{i1}$ >83% for ketones and between 64 and 85% for alcohols), only except for 2-nonanone with $\%Condens_{i1}$ of 35 %.

At the same permeate pressure value of 1500 Pa and selecting $T_{1,condens}$ [°C] of 10° C (second vertical line in Figure 5 c), an almost total absence of all off-flavours was achieved in the first condenser (with $Condens_{i1}$ <10 % sulphur compounds and <20 % of aldehydes). The implementation of this condensation temperature would, however, lead to a reduction of the desirable aromas captured in the first condenser, recovering just about 40-65 % of each aroma. Therefore, to obtain aroma concentrates with desirable organoleptic properties and free from off-flavours, we propose for this case-study to operate at a permeate pressure of 1500 Pa and with a $T_{1,condens}$ of 10 °C.

This pressure condition (1500 Pa) allows for good selectivity against the main off-flavours, which leads to more efficient recovery of desirable aromas from the seafood effluent with good removal of the off-flavours. Concerning the selected temperature of the first condenser at 10 °C, it allows for a low condensation of off-flavours, lower than 10 % for DMTS present in the model solution of seafood aromas. It also allows the recovery of 40-60 % of alcohols and ketones, except for 2-nonanone.

2.2.6. Conclusions

The mathematical model discussed and validated in this work allows, with a minimum of experimental work (total condensation experiments and determination of inert gas molar flow rate) and the use of reliable thermodynamic properties, to simulate the integrated process of pervaporation with fractionated condensation. Even at different permeate pressures, this model allows for assessing whether a separation between a target valuable aroma and an off-flavour is feasible or not, and which are the most appropriate conditions to achieve optimal separation, with reduced experimental work.

The sensitivity analysis performed in this work emphasises the need for an accurate estimation of relevant thermodynamic parameters, in particular the saturated vapour pressure of the compounds present in the feed stream.

The modelling approach discussed and validated in this work represents a simple tool that might be used in diverse integrated processes of pervaporation – fractionated condensation, requiring minimum experimental information, allowing to optimise the process operating conditions to achieve a target composition of condensates.

2.3. Recovery of valuable aromas from sardine cooking wastewaters by pervaporation with fractionated condensation: Matrix effect and model validation

Submitted as: M. João Pereira, Manuela Pintado, Carla Brazinha and João Crespo, " Recovery of valuable aromas from sardine cooking wastewaters by pervaporation with fractionated condensation: Matrix effect and model validation", Membranes (2022).

The author was involved in planning and performing the experiments, as well as in the discussion and interpretation of the results and preparation of the manuscript.

2.3.1. Summary

Due to the lack of studies addressing the influence of real food matrices on integrated organophilic pervaporation/fractionated condensation processes, the present work analyses the impact of the real matrix of sardine cooking wastewaters on the fractionation of aromas. In a previous study, a thermodynamic/material balance model was developed to describe the integrated pervaporation - fractionated condensation process of aroma recovery from model solutions that emulate seafood industry aqueous effluents, aiming to define the best conditions for off-flavour removal.

This work assesses whether the previously developed mathematical model, validated only with model solutions, is also applicable to predict the fractionation of aromas of different chemical families from real effluents (sardine cooking wastewaters), aiming for off-flavours removal. It was found that the food matrix has no influence with substantial detrimental consequences on the model simulations, which validates and extends the applicability of the model.

2.3.2. Introduction

The large majority of studies performed for aroma recovery by pervaporation have been accomplished using model solutions [115]. The use of model systems is effective for a simple and detailed analysis of process performance and optimisation. However, model solutions cannot reproduce all the complex variety of constituents of the feed stream, with diverse concentrations, and chemical and organoleptic properties, which contribute to the overall aroma profile [116]. The pervaporation of real feed mixtures should also be studied because the concentration of volatiles is usually lower than in model solutions, due to potential interferences of lipids and proteins in the aroma profile [48], which is mostly neglected when studying model solutions.

In a previous study, Pereira et al. [117] proposed a mathematical model for the pervaporation-fractionated condensation aiming at the recovery of aromas free from off-flavours using

a model solution that mimicked seafood cooking wastewaters. This model allows simulating the mass and composition of each compound in the condensers arranged in series, mousing as input information the permeate fluxes of each aroma under study (obtained experimentally), operating conditions used in the process, and thermodynamic parameters of each aroma. The model is based on mass balances and thermodynamic equilibrium in each condenser.

For many years, the production of commercial seafood flavourings used solid by-products. Nowadays, seafood cooking water has emerged as a promising source for producing "natural-like" aroma concentrates, valuable for the food and feed market sectors [97]. In this work, the fractionation and separation of desirable target aromas from off-flavours are explored benefiting from both the membrane intrinsic selectivity and the selectivity of fractionated condensation consecutive steps.

The main objective of this work is to study the effect of the matrix in the aroma recovery from sardine cooking wastewaters by the integrated process of organophilic pervaporation/ fractionated condensation, assuring off-flavour removal. Concretely, the objective is to validate the mathematical model previously developed for model solutions, extending it to application for a real matrix, a complex sardine cooking wastewater. If the model correctly predicts the fractionation of the different aromas, despite the complexity of the real solution, the applicability of the model will increase significantly, opening opportunities for use with other real matrices

2.3.3. Materials and methods

The sardine cooking wastewater was kindly provided by the company A Poveira S.A. (Laúndos, Portugal). This effluent is the result of steaming the fish for 7 min at 100 °C. An acorn extract with antioxidant properties was added to the sardine cooking wastewater at the outlet of cooking chambers at a 1% (v/v) concentration to prevent lipid oxidation and suppress aroma deterioration. The effluent was collected, transported, and stored at -20 °C until needed.

The experimental setup and analytical methods of study were the same as described in previous studies [117], [118].

A radial flow flat module (GKSS, Germany) was employed, presented, and discussed in detail in Schafer [34]. The membrane used was a PervapTM 4060 (DeltaMem AG, Switzerland), an organophilic dense membrane, with a membrane area of 10^{-2} m2. The active layer of polydimethylsiloxane (PDMS) was shown to have an excellent performance for the permeation of organic compounds by pervaporation, as well as a good affinity for seafood aromas [103], [104].

The operation conditions applied in this study were the optimised conditions obtained previously in the studies performed with a model solution [117]. According to these, the permeate pressure applied was 1500 Pa. The temperature of the first condenser $T_{1,condens}$ was set at -10 °C, and the temperature of the second condenser $T_{2,condens}$ was at -196 °C.

At the end of the trials, the membrane used was rinsed with a known amount of water at room temperature and the content of lipids, proteins, and aromas present in this solution was characterised according to the methods described in Pereira et al., [118].

2.3.4. Results and discussion

2.3.4.1. Characterization of Sardine Cooking Wastewaters

Alcohols, aldehydes, and ketones are part of the aroma profile of the sardine cooking wastewaters, as revealed by solid-phase microextraction followed by gas chromatography-mass spectrometry (SPME/GC-MS). The overall aroma profile of sardine cooking wastewaters is presented in **Table 2.9** (See section 2.1.4.1.), and it is identical to the aroma profile of sardines investigated by other researchers [79], [80]. Some chemical markers were selected to study the effect of the matrix in this process, which are 1-penten-3-ol and 1-octen-3-ol, as alcohols; heptanal, 2,4-heptadienal, (E,E)-, 2,6-nonadienal, (E,Z)-, as aldehydes and 2-nonanone as ketone. These chemical markers were selected based on the main groups of chemicals present in sardine cooking wastewaters, with diverse organoleptic properties. The main compound present in higher concentrations was 1-penten-3-ol.

Table 2.9. Aroma compound identified in Sardine Cooking wastewaters

Aroma compounds	Area*	[C_i] (ppm)*	Aroma compounds	Area*	[C_i] (ppm)*
Aldehydes			**Alcohols**		
Hexanal	123246866		1-Penten-3-ol	79964041	0.100
Heptanal	28381616	0.006	1-Octen-3-ol	181778114	0.008
2-Hexenal, (E)-	58129859		Octa-1,5-dien-3-ol, (5Z)-	86516933	
Octanal	16922895		2-Ethylhexanol	7501041	
Nonanal	79342642		1-Octanol	590437999	0.100
2-Octenal, (E)-	39611501		1-Penten-3-ol	79964041	0.008
2,4-Heptadienal, *(E,E)*-	88078708		1-Octen-3-ol	181778114	
2-Nonenal, (E)-	21644129	0.011	**Ketones**		
2,6-Nonadienal, *(E,Z)*-	92347778	0.044	2-Nonanone	44929994	0.001
2-Decenal, (E)-	12169097		3,5-Octadien-2-one	113901449	
Acids			2-Undecanone	8019036	
Hexanoic acid	89583410				

Mean values of integration peak area for all compounds identified and concentration (ppm) of chemical markers. The aromas were identified by comparing their retention indices relative to C8–C20 n-alkanes and mass spectra to those in the NIST Library Database. Quantification was done with calibration curves of the pure standards, evaluated under the same circumstances. Underlined compounds are off-flavours

2.3.4.2. Pervaporation-fractionated condensation processing of sardine cooking wastewaters

The permeate was generated through pervaporation experiments with sardine cooking wastewaters under upstream operating conditions described in the previous section. The total permeate fluxes obtained in seafood model solution experiments using the same operating conditions was 889.84 g/m2.h and with sardine cooking wastewaters was 731 g/m2.h. This lower value of permeate flux was expected due to the total lipid (28.13 ± 2.84 g/100g) and protein content (25.38 ± 1.95 mg/ml) of the sardine wastewater sample [118]. The presence of lipids and proteins in the feed medium might lead to interactions with aroma compounds present and also to some degree of fouling of the pervaporation membrane.

Table 2.10 shows the individual fluxes [mol/(m^2.s)] and the permeabilities [mol/(m.s.Pa)] to the aromas under study, as well as the separation factor (calculated against water).

Table 2.10. Experimental parameters of pervaporation performed with a downstream pressure of 1500 Pa, with real wastewater : aroma flowrate (*Ji*), permeability (*Li*) and selectivity of each aroma (against water).

Compound	J_i [mol/m².s]	L_i [mol/(m.s.Pa)]	*Separation factor [-]*
Sardine cooking wastewater			
1-Penten-3-ol	**$6.58x10^{-7}$ ±$8.64x10^{-9}$**	**$6.25x10^{-11}$±$4.15x10^{-12}$**	**4.20±0.28**
1-Octen-3-ol	$3.91x10^{-8}$±$1.66x10^{-9}$	$2.33x10^{-11}$±$7.95x10^{-13}$	11.19±0.38
2-Nonanone	$1.89x10^{-8}$±$9.67x10^{-10}$	$1.48x10^{-11}$ ±$9.17x10^{-13}$	51.03±3.14
Heptanal	$2.80x10^{-8}$±$9.56x10^{-10}$	$3.73x10^{-12}$±$3.06x10^{-13}$	7.31±0.60
(*E*2, *Z*6)-Nonadienal	$4.65x10^{-7}$±$2.86x10^{-8}$	$1.48x10^{-11}$±$9.17x10^{-13}$	171.15±19.73

Data presented are the mean±s.d. values. Underlined compounds are off-flavours

The important and main alcohol 1-penten-3-ol, responsible for the aroma of fresh marine products, is generated from polyunsaturated fatty acids [79]. 1-Penten-3-ol presents the highest values of individual flux (*Ji*) and permeability (*Li*). However, the off-flavour (*E2, Z6*)-nonadienal shows a close permeate flux and the highest separation factor, which reinforces the importance of conjugating fractionated condensation to the pervaporation process to enable the off-flavour removal.

At the end of the process, to better understand the effect of the matrix in the pervaporation process, the content of total proteins and lipids that remained adsorbed to the membrane, as well as the aroma content in this adsorbed layer, were analysed by Lowry and Bligh & Dyer methods, respectively. There was no gel formation on the membrane surface and indeed the protein content in the membrane was quite residual (6.15-8.46 μg/m²), only slightly more relevant in terms of lipids showing 1.3-2 μg/m². Concerning the aromas, a small number of aromas remained in the membrane in a very small concentration, only 2-nonanone and 1-octen-3-ol were found in residual concentrations of 10 and 20 μg/m², respectively.

2.3.4.2.1. .Model validation with experimental values

The thermodynamic/material balance model was developed to simulate the recovery of aromas at a given permeate pressure employing fractionated condensation with two condensers in series, supported by an efficient and optimized fractionated condensation (see the complete explanation by Pereira et al. [117]. In short, starting from simple experimental inputs as i) permeate flux of each aroma present in the system, ii) thermodynamic parameters (for each compound in the feed: Antoine constant and activity coefficient at infinite dilution), and iii) operation conditions of downstream pressure and temperature, it is possible to simulate the composition of the condensates obtained in the sequential condensers. Through a system of equations that describe the thermodynamic equilibrium conditions and with the support of required material balances, we can select the best-operating conditions to achieve the best separation of desirable flavours from off-flavours. In the end, the expressions for calculating

the percentage of condensation of water and aroma(s) in the first condenser are obtained, respectively, by Eqs. (20) and (21).

$$\%condens_{w1} = 1 - \frac{n_{inert}}{n_{w0}} \cdot \frac{p_{vw(T1,condens)}}{p_{perm} - p_{vw(T1,condens)}} \quad (20)$$

$$\%condens_{aroma1} \cong 1 - \frac{n_{inert}}{n_{aroma0}} \cdot \frac{\varkappa_{aroma1} \cdot \gamma^{\infty}_{aroma1} \cdot p_{varoma(T1,condens)}}{p_{perm} - p_{vw(T1,condens)}} \quad (21)$$

where n_{inert} is the inert gas molar flow rate in the stream, P_v is the saturation vapour pressure of water or aroma, p_{perm} is the permeate pressure applied to the system, $n_{w \text{ or aroma}0}$ is the molar flow rate before the first condenser, $\varkappa_{w \text{ or aroma}}$ is the molar fraction in feed and $\Upsilon^{\infty}_{aroma}$ is the infinite activity coefficient of the aroma.

The model developed was applied for a permeate pressure of 1500 Pa, where the percentage of compound i that is condensed/recovered in the first condenser, $\%Condens_{i1}$, was predicted for different values of $T_{1,condens}$ [° C].

Figure 2.19 shows the simulations obtained for each aroma present in the sardine cooking wastewater and the experimental values acquired, in terms of $\%Condens_{i1}$ (the fraction of each chemical compound i that condenses in the first condenser) versus the temperature of the condenser, $T_{1,condens}$.

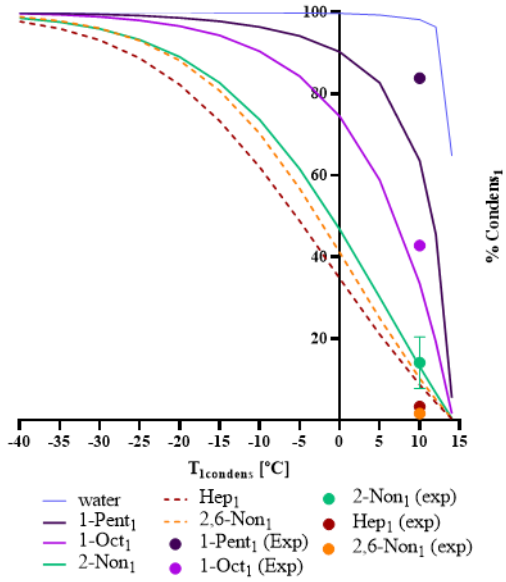


Figure 2.19. Model simulation obtained for the sardine cooking wastewaters with experimental validation for five different aromas presents. Percentage of condensation of each compound (water, 1-penten-3-ol, 1-octen-3-ol, heptanal, 2,6-nonadienal and 2-nonanone) in the 1st condenser ($\%Condens_{i1}$) as a function of the temperature of the same condenser ($T_{1,condens}$). Operating conditions: Pervap 4060 membrane; T_{feed}=60° C; p_{perm} =1500 Pa; Lines refer to simulated values and symbols to experimental data, which was analysed in triplicate.

Figure 2.19 reveals a good adherence between the experimental and the simulated results of $\%Condens_{i1}$ as a function of the temperature of the condenser and, consequently, for the

composition of condensates. This result means that, although the real medium composition is much more complex than the model solution previously studied, it is not necessary to modify the thermodynamic/material balance model used, which can be applied with success to evaluate if a given fractionation of aromas (such as the fractionation between target aromas and off-flavours) can be achieved.

Under the experimental conditions used in this work, 1500 Pa of permeate pressure and $T_{1,condens}$ [°C] of 10° C (see Figure 2.19), a good off-flavour removal was achieved with partial retention of off-flavours in the 1st condenser lower than 3% for heptanal and 1.6% for (*E2, Z6*)-nonadienal. These retention values correspond to an off-flavours concentration of 0.02 and 0.67 $mg_{off\text{-}flavours}/Kg_{condensate}$ of heptanal and (*E2, Z6*)-nonadienal, respectively, in the 1st condensate. In a conclusion, in terms of aroma quality, the condensate recovered in the first condenser is reduced in off-flavours. Both off-flavours concentrations are below their threshold (limit of human olfactive perception) of 0.60 and 0.70 mg/L of heptanal and (*E2, Z6*)-nonadienal, respectively). On the other hand, it should be recognised that the recovery of desirable aromas in the first condenser is not complete, not assuring off-flavour removal: 84% of 1-penten-3-ol is recovered in the first condenser but 1-octen-3-ol is recovered by 43% and the ketone 2-nonanone is only recovered by 14%.

This model proves to be an excellent tool to simulate the percentage of condensation of each aroma in each condenser at a particular downstream pressure and condenser temperature, as well as the resulting condensate composition. It enables the comparison and definition of fractionated condensation procedures based on the goal of a given industrial process.

2.3.5. Conclusions

The integrated process of pervaporation – fractionated condensation has proven to be a potential approach for the valorisation of canning industry effluents by the recovery of valuable aromas.

The model used shows a good match between experimental and predicted values, despite the high heterogeneity of the sardine cooking wastewater. The effect of the matrix did not demonstrate significant negative effects on the model simulations, making possible its use without further modification.

The model applied and validated for sardine cooking wastewaters, proved to be a useful tool to predict the fractionation of aroma compounds, here illustrated by the removal of off-flavours to obtain aroma products with potential commercial value. Additionally, due to the range of chemical families evaluated, this model represents a tool that might be easily applied to other real matrices, aiming the recovery of surplus aromas in a circular economy perspective.

3.
INTEGRATED SYSTEMS FOR THE PRODUCTION OF RED WINE AROMAS

3.1. Valorisation of the model headspace of red wine fermentation: comparison of an integrated vapour permeation-modelling fractionated condensation and modelling fractionated condensation process for off-flavour reduction

Published as: M. João Pereira, António Ferreira, Carla Brazinha and João Crespo, "Modelling of Fractionated Condensation for Off-Flavour Reduction from Red Wine Fermentation Headspace", Membranes (2022), 12(9), 875.

The author was involved in planning and performing the experiments, as well as in the discussion and interpretation of the results and preparation of the manuscript.

3.1.1. Summary

A mathematical model of fractionated condensation is proposed for predicting the recovery and fractionation of target aromas from red wine fermentation headspaces to remove off-flavours. The applicability of the model is assessed for two different alternative processes: fractionated condensation and vapour permeation–fractionated condensation. The aromas of the headspace of red wine fermentation are commonly lost through the fermenter venting system and are enhanced by the stripping effect of the produced CO_2. To mimic the operating conditions during the red wine fermentation, all experiments were performed at 30 °C with a red wine model solution containing relevant red wine aromas, the cosolvent ethanol at representative concentrations, and CO_2. Both studied processes allow for a good recovery of esters in the 2nd condenser, with over 80% of ethyl acetate and isoamyl acetate recovery when using vapour permeation–fractionated condensation and a recovery of 84–96% of all esters when

using fractionated condensation. However, only the integrated process of vapour permeation–fractionated condensation achieves a significant decrease in the amount of ethyl phenols (off-flavours compounds) in the 1st condenser, above 50%, as expected due to the use of an organophilic membrane. The developed model was validated experimentally for the integrated process, proving to be a highly valuable tool for the prediction of aroma fractionation, aiming at the removal of off-flavours.

3.1.2. Introduction

One of the most important wine quality indicators is its aroma profile, which comprises hundreds of distinct components that are responsible for the wine's flavour and odour [59], [68]. The food flavour industry is expected to be worth 20.12 billion US dollars by 2028 [119]. Several alcoholic beverages are available on the market, including wine, beer, cider, and spirits, with the world's top players accounting for more than 60% of global sales [62].

The presence of off-flavours in an alcoholic beverage may lead to a consumer's perception of inferior quality, which can be extremely costly to the industry [66]. Volatile phenols, particularly 4-ethyl phenol and 4-ethyl guaiacol, are aroma molecules that, at the perception threshold limit, compromise wine quality by imparting aroma defects such as "horse sweat," "animal," "leather," and "medicinal" [68]. According to the literature, preventing the generation of volatile phenols by *Brettanomyces* is the major problem in contemporary winemaking and is to blame for considerable economic losses globally [120], [121].

The synthesis of these compounds, which often happens during fermentation by *Brettanomyces/Dekkera bruxellensis*, results in the wine characteristic known popularly as "Brett-taint" [67]. Off-flavour defects caused by the presence of these molecules are one of the most common organoleptic issues encountered during the production of many fermented alcoholic drinks (e.g., wine, beer, cider, etc.) [66].

The preservation of wine aroma during processing is also a critical topic in food technology that is becoming increasingly important. The demand for high-quality and widely characterised products to compete in the global market through differentiation has become imperative in many industries [122]. The amount of volatile compounds produced at the end of the fermentation process is mostly controlled by yeast synthesis, while it is also influenced by depletion due to the CO_2 stripping effect, which drags aromas to the fermenter headspace, where they are lost by venting. During wine fermentation, huge amounts of CO_2 (up to 40 L/L of must) are rapidly emitted, resulting in a continuous stripping-off of volatile compounds, with up to 70% of the produced volatile compounds being stripped away [61], [123]. To minimise the resultant deterioration of the final aroma bouquet, these aroma compounds should be recovered and reintroduced into the final product [9].

Condensation can be performed at different temperatures in a sequence of condensation steps, aiming to achieve fractionated condensation and separate fractions enriched with target

compounds. The temperature of each condenser must be controlled in accordance with the downstream pressure and its separation and recovery characteristics [23]. The various components are condensed differently, according to operating conditions and the liquid–vapour equilibrium [36].

Mathematical models of fractionated condensation were previously studied when this operation was integrated with pervaporation. Pervaporation-fractionated condensation processes from wine model solutions were investigated by Brazinha et al. [10]. A mathematical model was developed for the fractionated condensation step to evaluate the influence of the non-condensable gas CO_2 and the cosolvent ethanol on the recovery of aroma condensates from aqueous and hydroalcoholic solutions. Pereira et al. [117] studied pervaporation-fractionated condensation from by-products of the seafood industry to remove their off-flavours. These mathematical models of fractionated condensation require information about feed composition, are based on mass balances, and assume thermodynamic equilibrium. If this assumption is not valid, the model cannot predict the composition of each condenser. In this work, the fractionated condensation model is extended to two different processes—a standalone fractionated condensation and an integrated vapour permeation/fractionated condensation—aiming at the recovery of red wine aromas free from off-flavours.

3.1.3. Experimental

3.1.3.1. Materials

In the vapour permeation experiments, the hydrophobic dense membrane PervapTM 4060 (DeltaMem AG, Allschwil, Switzerland) was used. It is a commercial flat sheet membrane with a polydimethylsiloxane (PDMS) active top layer with a thickness of 2 μm and an effective membrane area of 10−2 m2. The solvents used in the model solutions were mineral carbonated water (brand Vimeiro, Águas do Vimeiro S.A., Maceira, Portugal) and ethanol (Panreac, Barcelona, Spain, 99.5% purity). The five aromas chosen as model components were ethyl acetate (Sigma–Aldrich, Missouri, USA, ≥99% purity), ethyl hexanoate (Sigma–Aldrich, Missouri, USA, ≥99% purity), isoamyl acetate (Sigma–Aldrich, Missouri, USA, ≥97% purity), 4-ethyl phenol (Sigma–Aldrich, Missouri, USA, ≥99% purity), and 4-ethyl guaiacol (Sigma–Aldrich, Missouri, USA, ≥98% purity). A DVB/CAR/PDMS fibre, 50/30 μm, 2 cm, and a Sapiens, Wax-MS (TeknoKroma, Barcelona, Spain) were used for SPME/GC-MS analysis.

3.1.3.1.1. Definition of the Model Solution of the Red Wine Fermentation Headspace

A model solution was defined, mimicking a red wine fermentation headspace, prepared with mineral carbonated water (brand Vimeiro, Águas do Vimeiro S.A., Maceira Portugal), which was further supersaturated with carbon dioxide (Air Liquide, Paris, France, 99.95%

purity) administrated at a flow rate of 100 L of CO_2 $L^{-1}.h^{-1}$, and ethanol (Panreac, Barcelona, Spain, 99.5% purity) was added at 10% *wt* in water.

The aroma compounds were chosen considering the most common esters present in red wines and the off-flavours generated during the vinification process. The properties of each chosen aroma of the model solution are listed in **Table 3.1.A**. The thermodynamic parameters and the organoleptic qualities are summarised in **Table 3.1.B**, which includes activity coefficients at infinite dilution and saturation vapour pressure.

Table 3.1. Composition of the model solution of red wine: (**A**) characterisation of the selected red wine aroma compounds and (**B**) the physicochemical properties of the aroma compounds)

(A)

Aroma compound	Family of chemicals	Reason to include the aroma in the model solution	Concentration of the model solution
Ethyl acetate	Esters	Esters are the most abundant aromas produced by wine yeasts. Ethyl acetate, isoamyl acetate and ethyl hexanoate are considered the main component of a fruit flavour [124]	50 ppm
Ethyl hexanoate			8 ppm
Isoamyl acetate			6 ppm
4-ethyl phenol	Phenols	When volatile phenols reach certain concentrations affects the quality of the wine revealing aroma defects normally described as "horse sweat", "animal" and "medicinal" [120]	0.6 ppm
4-ethyl guaiacol			1 ppm

*The odour threshold is 440 ppb for 4-EP and 33 ppb for 4-EG [125].

(B)

Compound	MW (g/mol)	BP (°C)	$P^{vi}_{25°\,C}$ (Pa)	$\Upsilon^{\infty}_{25°\,C,\,water}$	$\Upsilon^{\infty}_{25°\,C,\,10\%Etoh}$
Ethyl acetate	88.11	77.10	12622.12	50	37
Ethyl hexanoate	144.21	167.00	49898.73	12615	9014
Isoamyl acetate	130.18	142.50	1470959.90	3865	2280
4-ethyl guaiacol	152.19	236.50	7.56	8383	
4-ethyl phenol	122.16	217.90	33.19	23742	

(MW = molecular weight, BP = boiling point, pvi = saturation vapour pressure, $\Upsilon\infty$ = activity coefficient at infinite dilution). *For ethyl phenols, the activity coefficient of the aromas in a mixture with 10% ethanol was not calculated due to the lack of information about the activity coefficients at 70 °C in water

The activity coefficient values presented for aqueous solutions were found in the literature [10], [34]. The influence of ethanol on the aroma activity coefficient is well discussed in the literature, resulting in a decrease in these values [10], [106]. The Pierotti modified parameters obtained by Eq. 22 were used to calculate the activity coefficient of the aromas in a mixture with 10% ethanol:

$$\gamma^{\infty}_{25\,°C,10\%\,Etoh} = \gamma^{\infty}_{25\,°C,water} \cdot \left[\frac{\gamma^{\infty}_{70\,°C,10\%\,Etoh}}{\gamma^{\infty}_{70\,°C,water}}\right] \quad (22)$$

where the activity coefficients at 25 °C in water and 70 °C, both in water and in a 10% ethanol aqueous solution, were determined in [34].

3.1.3.2. Experimental unit

3.1.3.2.1. Fractionated Condensation with CO_2 Stripping Gas Experiments

The experimental setup, presented in Figure 3.1, was built to recover aromas by CO_2 stripping without the assistance of a membrane, followed by fractionated condensation. This setup consists of a feed vessel with CO_2 supersaturation by the injection of gas inside the model solution, which aims to mimic the wine fermentation vessel. The vapour stream was produced in the same way as in the integrated vapour permeation–fractionated condensation. All the tubing was stainless steel, and the fractionated condensation approach included two U-shaped glass trap condensers in series. The first condenser was refrigerated using an FP 500-MC (Julabo, Seelbach, Germany), and liquid nitrogen was used to submerge the second condenser.

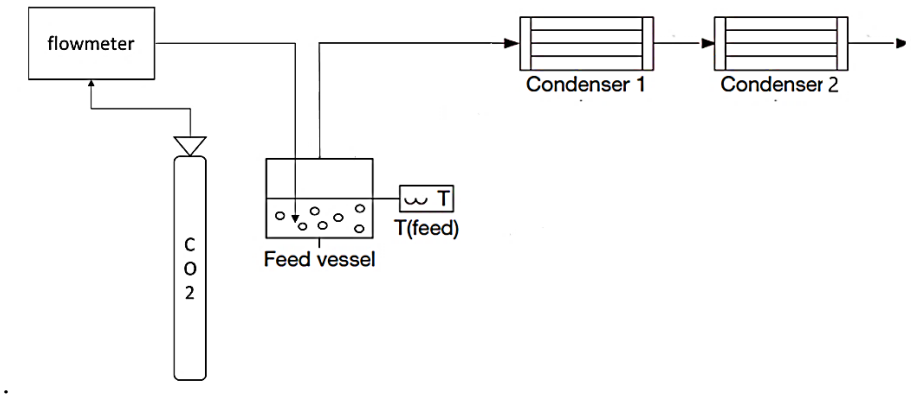


Figure 3.1. Diagram of the experimental setup of fractionated condensation with CO_2 stripping gas with two condensers connected in series.

3.1.3.2.2. Vapour Permeation with CO_2 Stripping Gas and Fractionated Condensation

Figure 3.2. illustrates the experimental setup used, which was planned to recover aromas using vapour permeation with CO_2 stripping gas. A CO_2 bottle was linked to a flowmeter, and CO_2 was injected into the model solution. Working at a constant CO_2 flow rate of 100 L of CO^2 $L^{-1}{\cdot}h^{-1}$ and a feed temperature of 30 °C, a vapour stream was generated, emulating the vapour stream generated in a red wine fermenter. A radial flat module (GKSS, Geesthacht, Germany) was tested, which was described in detail in [34]. The upstream tubing was either Viton or Teflon, and the metal used in the unit was stainless steel. In this study, an E2M5

rotary vane dual-stage mechanical vacuum pump (Edwards, Burgess Hill, UK) ensured vacuum conditions on the permeate side, with a p_{perm} of 1000 Pa. The apparatus was equipped with a TPR280 pressure gauge (Pfeiffer Vacuum, Aßlar, Germany), which collected permeate pressure measurements of p_{perm} (Pa). The downstream pressure, p_{perm} (Pa), was regulated by an RVC 300 pressure controller (Pfeiffer Vacuum, Aßlar, Germany), which varied the resistance produced by an RME 005 electro valve (V2) (Pfeiffer Vacuum, Germany). The downstream circuit incorporated two condensation U-shape glass trap condensers in series, using an FP 500-MC (Julabo, Seelbach, Germany) to refrigerate the first condenser. Liquid nitrogen was used to submerge the second condenser.

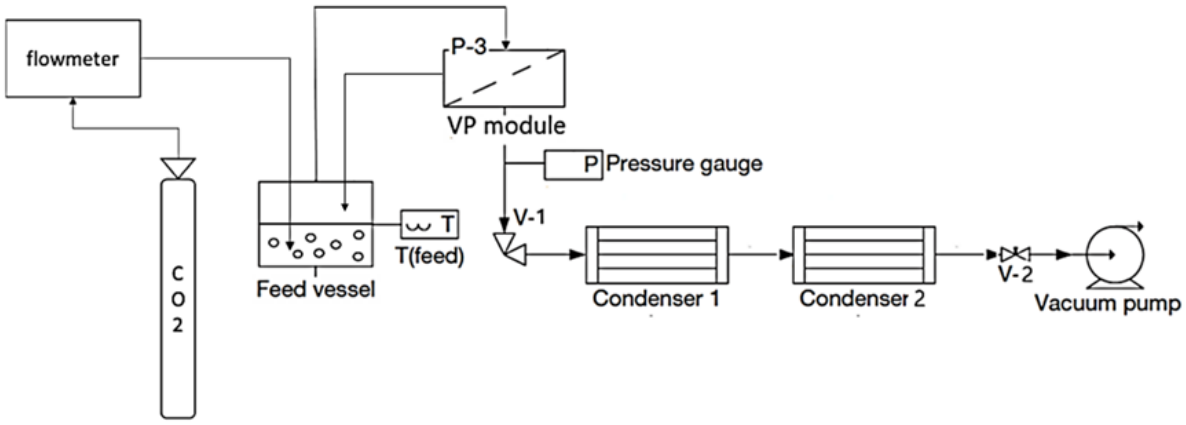


Figure 3.2. Diagram of the experimental setup of a vapour permeation apparatus with two condensers connected in series.

3.1.3.3. Operating conditions

3.1.3.3.1. Feed compartment

A volume of 3 L of the model solution with aromas of red wine (see Section 3.1.3.1.1.) was placed in the vessel with a headspace of 2 L (See Figure 3.3). The runtime of each trial was established at 3 h, based on the ratio between the membrane area of 10^{-2} m^2 and the volume of the feed vessel. Considering the limited experimental time, previous membrane conditioning was performed by permeating ethanol at 10% *wt* in mineral carbonated water for 4 h before each vapour permeation experiment. The temperature of the feed vessel was kept constant at 30 °C throughout all experiments.

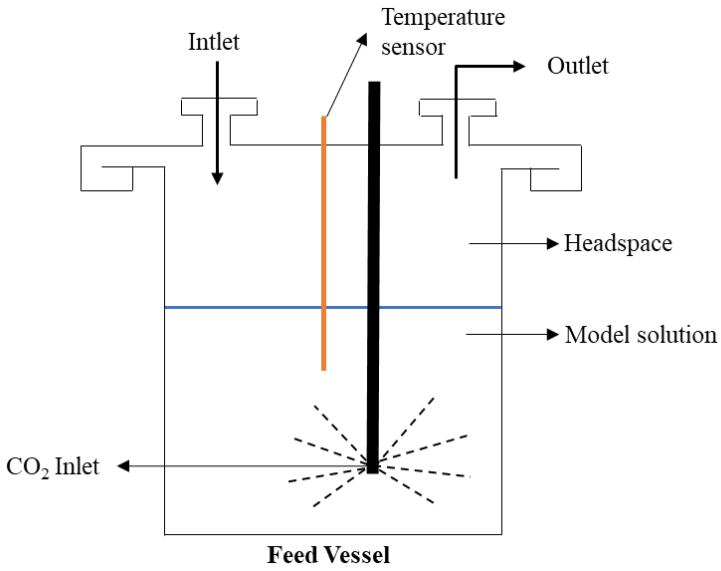


Figure 3.3. Scheme of the feed vessel

3.1.3.3.2. Fractionated Condensation Experiments

In the fractionated condensation experiments, the following parameters were controlled: the temperature of the feed stream T_{feed} was maintained at 30 ± 1 °C, and the CO_2 flow rate was kept constant at 100 L of CO_2 L^{-1} h^{-1}. The temperature of the first condenser, $T_{1,condens}$, was set at −40 °C, and the temperature of the second condenser, $T_{2,condens}$, was −196 °C. The experiments were performed at atmospheric pressure.

3.1.3.3.3. Vapour Permeation–Fractionated Condensation Experiments

In the vapour permeation experiments, different parameters were controlled: the temperature of the feed stream T_{feed} was maintained at 30 ± 1 °C, and the CO_2 flow rate was kept constant at 100 L of CO_2 L^{-1} h^{-1}. Despite being higher than the CO_2 production rate of wine fermentation, this flow rate was selected to ensure a gas stripping effect on the aromas present in the model solution and to obtain a CO_2-supersaturated feed with an upstream pressure above atmospheric pressure. The temperature of the first condenser, $T_{1,condens}$, was studied at −40, −25, and −15 °C, while the temperature of the second condenser, $T_{2,condens}$, was set at −196 °C. The permeate pressure, p_{perm}, of 1000.0 ± 50.0 Pa was chosen to ensure a good trade-off between energy consumption and transport driving force (Brazinha et al. [10]). A downstream pressure of 1 kPa is commonly reported for pervaporation industrial processes.

3.1.3.4. Analytical methods

Analyses by SPME-GC-MS were carried out using an AOC-5000 Plus autosampler (Shimadzu, Kyoto, Japan) and a GC-MS-QP2000 (Shimadzu, Kyoto, Japan). A DVB/CAR/PDMS fibre, 50/30 µm, 2 cm, and a Sapiens Wax-MS (TeknoKroma, Barcelona, Spain) chromatographic column with 60 m × 0.25 mm i.d. × 0.25 µm was used. The fibre was subjected to a

temperature of 40 °C for 15 min, with 250 rpm agitation in the head space inside the hermetic vial containing 7 mL of the sample. After this, the compounds were desorbed in the injector at 250 °C for 10 min with a 1:10 split ratio. Helium, at 4 mL/min, was applied as a carrier gas, and the chromatographic programme was started at a temperature of 40 °C and kept for 5 min, increased by 5 °C/min up to 170 °C, increased by 30 °C/min up to 230 °C, and maintained for 4 min. The temperatures of the ionisation source and the detector were 245 °C and 250 °C, respectively. Detection was performed in the m/z 29–300 range. The analysis were performed in triplicate.

3.1.3.5. Modelling of Fractionated Condensation Step

A mathematical model is necessary to simulate the capture of each aroma in the two in-series condensers to achieve successful fractionated condensation and remove off-flavours from potentially important target aromas. Such a model should also allow users to identify the best-operating temperatures in each condenser to separate off-flavours from desirable aromas. Supported by the work carried out by Brazinha et al. [10], a simple mathematical model for designing a fractionated condensation system, comprised of a set of condensers, can be established to achieve off-flavour removal [117].

The model is built on the assumption that the vapour stream in a series of condensers (non-total condensation) is in thermodynamic equilibrium with the liquid condensate phase in each condenser. This model uses the feed composition and feed flow operating parameters as well as the temperature of the first condenser as input variables (see **Figure 3.4**). The second condenser was assumed to achieve total condensation due to the low temperature of operation. The model simulation predicts the mass and composition of the condensate in each condenser. It is possible to simulate the composition of the condensates obtained in the condensers by starting with inputs such as: (i) the permeate flux of each aroma under study, (ii) thermodynamic parameters (saturation vapour pressure and activity coefficient at infinite dilution), and (iii) the operating conditions of pressure and temperature applied in upstream and downstream compartments. This is a very effective model for determining the optimal operating parameters for achieving the desired separation of valuable aromas from off-flavours

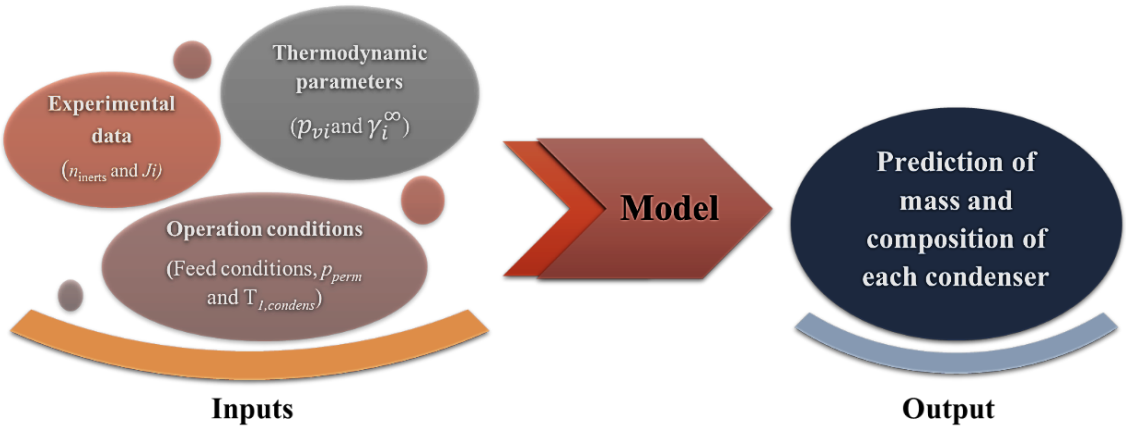


Figure 3.4. Diagram of the mathematical model applied in this work

For aqueous systems, the model was extensively explained in a previous paper [117]. Through a system of equations that constitute the model, we were able to select the best-operating conditions to achieve the best separation of valuable flavours from off-flavours. Equations (23) and (24) allow the determination of the percentage of water and aroma(s) condensation in the first condenser, respectively:

$$\%condens_{w1} = 1 - \frac{n_{inert}}{n_{w0}} \cdot \frac{p_{vw(T1,condens)}}{p_{perm} - p_{vw(T1,condens)}} \tag{13}$$

$$\%condens_{aroma1} \cong 1 - \frac{n_{inert}}{n_{aroma0}} \cdot \frac{\varkappa_{aroma1} \cdot \gamma^{\infty}_{aroma1} \cdot p_{varoma(T1,condens)}}{p_{perm} - p_{vw(T1,condens)}} \tag{24}$$

where n_{inert} is the inert gas (CO_2) molar flow rate in the stream, P_{vw} is the saturation vapour pressure of water or aroma, p_{perm} is the permeate pressure applied to the system, $n_{w\ or\ aroma0}$ is the molar flow rate of each species before the first condenser, $\varkappa_{w\ or\ aroma}$ is the molar fraction in the feed, and $\Upsilon^{\infty}_{aroma}$ is the activity coefficient at infinite dilution of the aroma(s).

It is important to highlight that this model can handle multi-component aroma systems because the target aroma compounds are extremely diluted in the feed solutions in most real-case scenarios. Therefore, each aroma behaves independently under these highly diluted aroma concentration conditions, with no flow coupling between them [107]. Furthermore, diluted aroma compounds do not affect water and ethanol transport [32].

This model was applied and experimentally validated for aqueous systems [117]. Following the strategy established for aqueous solutions, the model was developed for more complex solutions comprising ethanol and the presence of non-condensable gases.

$$x_{w1} + x_{et1} = 1 \tag{25}$$

$$x_{w2} = \frac{n_{w1,}}{n_{w1,} + n_{et1,}} \tag{26}$$

$$n_{w0} + n_{et0} = n_{T1} + n_{T2} \quad (27)$$

$$n_{w0} \cong x_{w1} \cdot n_{T1} + x_{w2} \cdot n_{T2} \quad (28)$$

$$\%condens_{i1} = 1 - \frac{n_{inerts}}{n_{i0}} \cdot \frac{x_{i1} \cdot \gamma_{i1} \cdot p_{vi}(T_{1,condens})}{p_{perm} - x_{w1} \cdot \gamma_{w1} \cdot p_{vw}(T_{1,condens}) - x_{et1} \cdot \gamma_{et1} \cdot p_{vet}(T_{1,condens})} \quad (29)$$

$i = w, et, aroma$

With Equations (23) and (24), it is possible to calculate the percentage of water and aroma(s) obtained in the first condenser. Due to the very diluted concentration of the aromas present in the stream, it was assumed that they did not affect the water and ethanol condensation. In Equation (26), it is considered a fact that the second condenser was used as a total condenser at a temperature of −196 °C. Considering this set of equations, a modified expression for the determination of the percentage of water, ethanol, and aroma(s) condensation in the first condenser is obtained in Equation (29).

The Antoine law equation was used to estimate the saturation vapour pressure of water, ethanol, and other aromas at the temperature range using the Antoine law constants shown in **Table 3.2**.

Table 3.2. Antoine law constants for each compound studied

Compound	A	B	C	Range of temperature (°C)	Reference
Water	5.40	1838.68	-31.74	-015 to 29.85	[108]
Ethanol	5.25	1598.67	-46.42	-0.15 to 78.55	[108]
Ethyl acetate	4.23	1245.70	-55.19	-	[108]
Ethyl hexanoate	15.99	3118.28	-106.76	-	[34]
Isoamyl acetate	16.50	2871.68	-110.92	-	[34]
4-Ethyl phenol	7.62	1955.30	195.46	99.76 to 244.80	[126]
4-Ethyl guaiacol	7.90	2203.80	234.22	85.27 to 233.09	[126]

For the estimation of the inert gases' molar flow, n_{inert} (mol/s), at the end of the vapour permeation trials, the vacuum pump was closed and the rising downstream pressure, p_{perm} (Pa), was monitored over time. The molar flow rate of inert gases is calculated by the ideal gas equation using the slope of the function $\Delta p_{perm}/\Delta t$ (Pa/s) above the saturation vapour pressure of water. At 30 °C, the obtained value of n_{inert} was $9.24 \times 10^{-6} \pm 2.00 \times 10^{-8}$ mol.s^{-1}. In the evaporation experiments at atmospheric pressure, the experimentally determined inert flux was 3.72×10^{-3}, equivalent to the CO_2 constant flow rate used.

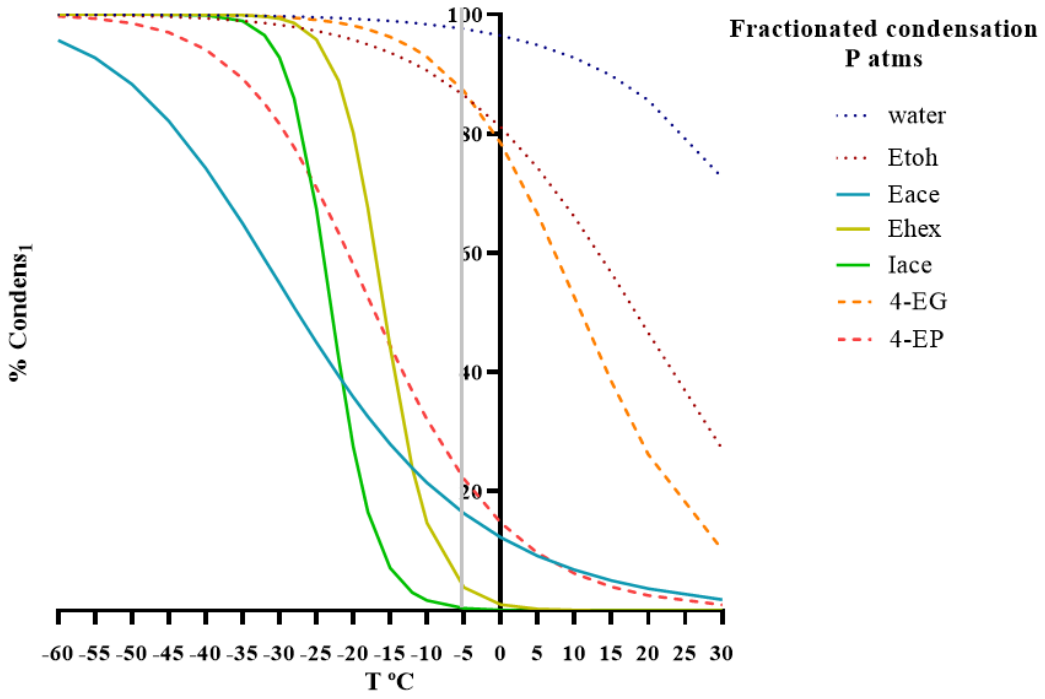
3.1.4. Results and discussion

3.1.4.1. Fractionated Condensation of the Model Solution of Red Wine

A model red wine solution was used under defined upstream operating conditions to originate a vapour that mimics the headspace of wine fermentation. The aromas were found in a range of concentrations in the feed vessel in the model solution (0.6–50.0 ppm). The vapour formed inside the feed vessel was the starting point of the model developed in this work.

To understand how effective the removal of off-flavours can be, modelling of the fractionated condensation process at atmospheric pressure was performed.

To achieve a reduction in the ethyl phenol content from the desirable aroma concentrate, the aroma fractionation aimed at obtaining two different condensates, where an optimal condensation temperature (in the first condenser) allowed the separation of valuable aromas from off-flavours. The mathematical model was used to simulate the percentage of each compound, *i*, recovered in the first condenser, $\%Condens_{i1}$, for different values of $T_{1,condens}$ (°C]), which are presented in Figure 3.5.



$T_{1,condens}$ [°C]	Yield of recovery in the 1st condenser						
	w	*Et*	*Eace*	*Ehex*	*Iace*	*4-EP*	*4-EG*
-5	98%	87%	16%	4%	1%	22%	87%

Figure 3.5. Model simulation of fractionated condensation for two different chemical families (esters and ethyl phenols) included in the red wine model solution. Percentage of condensation of each compound present (water, ethanol, ethyl acetate, ethyl hexanoate, isoamyl acetate, 4-ethyl phenol, and 4-ethyl guaiacol) in the 1st condenser ($\%Condens_{i1}$). Operating conditions: T_{feed} = 30 °C; p_{perm} = 101 kPa.

The optimal temperature suggested by the simulation was −5 °C. With this condition, the model estimated recovery of more than 87% of 4-EG, 22% of 4-EP, 16% of ethyl acetate, and a residual ($\%Condens_{i1}$ < 4%) of ethyl hexanoate and isoamyl acetate. These results represent a good recovery of the target esters in the 2nd condenser and a good reduction in the ethyl phenol content, more expressive in the case of 4-EG.

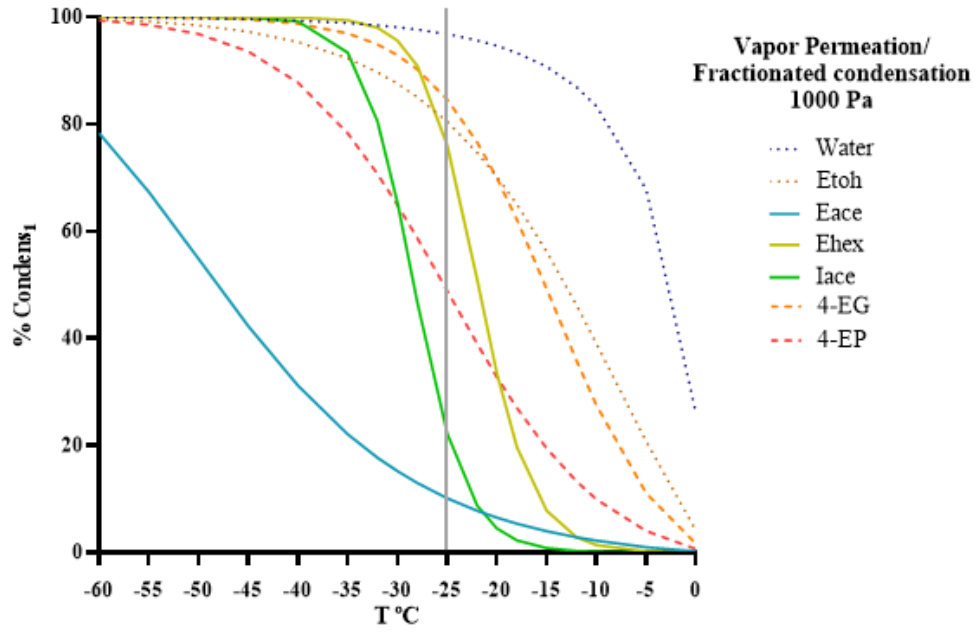
Due to the lower energy costs involved, fractionated condensation allows for aroma recovery under more economical conditions. However, this process does not allow for proper

off-flavour fractionation, leaving 78% of 4-EP, the most important off-flavour, in the 2nd condenser.

3.1.4.2. Vapour Permeation–Fractionated Condensation Processing of the Model Solution of Red Wine

The application of vapour permeation in the industry has been explored since the early 1980s, and it is now widely applied in the petrochemical and chemical sectors for the manufacture and purification of volatile organic compounds [127]. For this reason, it was considered relevant to test this model in an integrated vapour permeation/fractionated condensation process.

To find the best $T_{1,condens}$ (°C) to achieve the removal of the ethyl phenols for vapour permeation/fractionated condensation at a permeate pressure of 1000 Pa, a simulation was performed on the fractionated condensation process, and the $\%Condens_{i,1}$ was predicted for different values of $T_{1,condens}$ (°C). The obtained results are presented in **Figure 3.6**.



$T_{1,condens}$[°C]	Yield of recovery in the 1st condenser						
	w	*et*	*Eace*	*Ehex*	*Iace*	*4-EP*	*4-EG*
-25	97%	80%	10%	76%	22%	85%	49%

Figure 3.6. Model simulation of the integrated system of vapour permeation/fractionated condensation for two different chemical families (esters and ethyl phenols) included in the red wine model solution. Percentage of condensation of each compound present (water, ethanol, ethyl acetate, ethyl hexanoate, isoamyl acetate, 4-ethyl phenol, and 4-ethyl guaiacol) in the 1st condenser ($\%Condens_{i1}$). Operating conditions: Pervap 4060 membrane; T_{feed} = 30 °C; *pperm* = 1000 Pa.

Based on the simulation results, a temperature range of −25 to −15 °C may be suggested. The simulation showed that at a $T_{1,condens}$ (°C) of −15 °C, the 1st condenser ensures a residual

condensation ($\%Condens_{i1}$ < 8%) of all the esters present in the model solution as well as condensation of 20 and 50% of 4-EP and 4-EG, respectively. The model predicted retention of more than 80% for 4-EG, 75% for ethyl hexanoate, around 50% for 4-EP, and less than 20% for ethyl acetate and isoamyl acetate at a $T_{1,condens}$ (°C) of −25 °C. This represents a good recovery of some esters in the 2nd condenser and a good reduction in the ethyl phenol content. However, it implies the loss of a substantial part of the ethyl hexanoate content. The obtained results show that a complete separation of volatile phenols from the target esters is not possible by the proposed integrated process. However, it allows for a good reduction in the ethyl phenols.

Other research studies of fractionated condensation of wine aromas have shown similar behaviour concerning the condensation of esters. Brazinha et al. [10], aiming at the recovery and/or fractionation of aromas, studied the performance of vacuum-fractionated condensation integrated with an organophilic pervaporation process. Using a $T_{1,condens}$ (°C) of −9 °C, a similar $\%Condens_{i1}$ of 9% for ethyl acetate was obtained. In addition, Ribeiro et al. [9] studied aroma extraction by gas stripping in a bubble column followed by vapour permeation and compared it with the pervaporation process. By applying a multistage condensation system in series and a $T_{1,condens}$ (°C) of −30 °C, it was found that ethyl acetate remained in the vapour phase, leading to the recovery of pure water. The ester recovery was carried out in a second cold trap set to −117 °C. Regarding ethyl phenol removal, the only integrated system using membrane processes reported in the literature was a reverse-osmosis operation followed by a hydrophobic adsorptive resin, which showed significant reductions in 4-ethyl phenol and 4-ethyl guaiacol concentrations. However, there was a loss of other aroma compounds [68].

3.1.4.3. Model validation with experimental values

The values of $\%Condens_{i1}$ related to vapour permeation–fractionated condensation were simulated based on the model explained in the modelling of fractionated condensation step (Section 3.1.3.5. using the input parameters: $\varkappa_{i,\,feed}$, T_{feed}, p_{perm}, Ji, and n_{inert} and varying $T_{1,condens}$.). The red wine headspace model solution composition, including esters and ethyl phenols, is presented in **Table 3.1** (Section 3.1.3.1.1.). **Figure 3.7** illustrates the predicted and experimental percentages of recovery for each compound, *i*, in the first condenser. A good agreement between the experimental and simulated results of $\%Condens_{i1}$ was obtained. As described in Section 3.1.4.2., applying a $T_{1,condens}$ (°C) of −25 °C results in a reduction in the off-flavour concentration with the fractionated condensation.

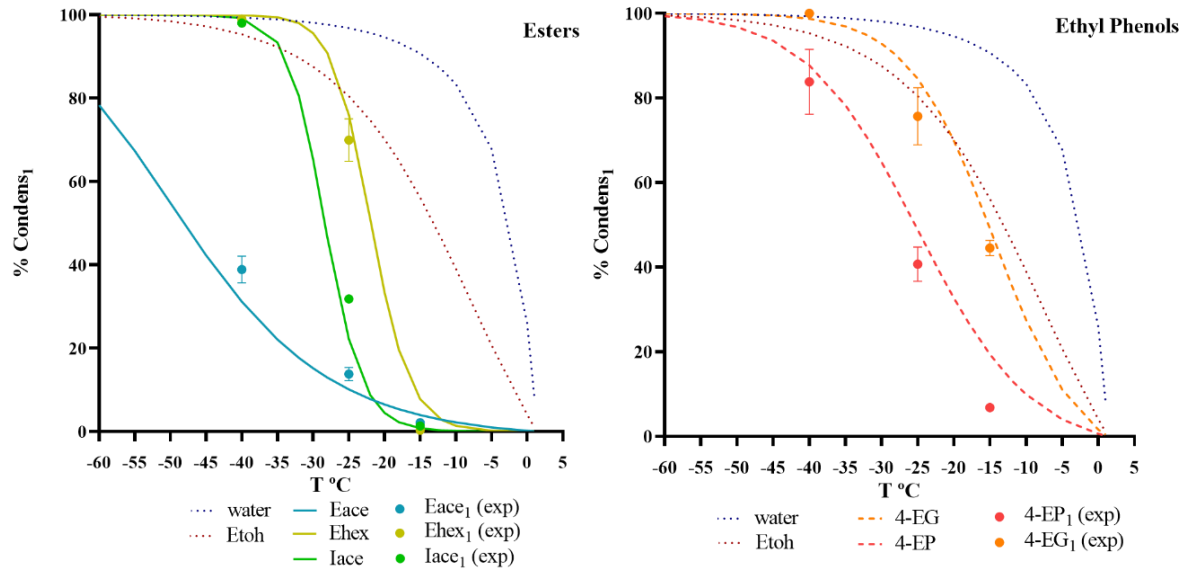


Figure 3.7. Validation of the model simulation for the different chemical families (esters and ethyl phenols) included in the red wine model solution. Percentage of condensation of each compound present (water, ethanol, ethyl acetate, ethyl hexanoate, isoamyl acetate, 4-ethyl phenol, and 4-ethyl guaiacol) in the 1st condenser ($\%Condens_{i1}$). Operating conditions: Pervap 4060 membrane; T_{feed} = 30 °C; p_{perm} = 1000 Pa; The symbols represent the experimental values, whereas the lines show simulated data.

Even in the presence of non-condensable gases, this model offers a simple and efficient approach for the simulation of the percentage of condensation of each compound in each condenser at a given downstream pressure and condenser temperature. For some of the aromas studied, the values of $\%Condens_{i1}$ were slightly overestimated, which was explained by a lower condensation efficiency due to the inert gas stripping effect, which could not be predicted thermodynamically.

3.1.5. Conclusions

Fractionated condensation, as a stand-alone step or in an integrated vapour permeation–fractionated condensation system, exhibited a good recovery of esters. However, only the vapour permeation–fractionated condensation system allowed for obtaining a significant decrease in the amount of ethyl phenols, with retention in the 1st condenser of over 80% for 4-EG and over 50% for 4-EP.

Even considering a complex feed solution, as in the case of a red wine fermentation headspace with ethanol and CO_2, this model proved that it might be applied as a quantitative tool to assess whether the separation between a target valuable aroma and an off-flavour is feasible and which conditions are most appropriate to achieve an optimal separation with minimal experimental work.

The modelling strategy outlined and validated in this study is a straightforward tool that can be applied to a variety of integrated processes. It requires little experimental data and

enables process operating conditions to be optimised to obtain the desired condensate composition.

4.
CONCLUSIONS AND FUTURE WORK

4.1. General conclusions

The scope of the work performed during this PhD project is the recovery and fractionation of aroma compounds from liquid or vapour effluents of the seafood and wine industries, aiming at producing innovative aroma profiles free of off-flavours. Different methods were addressed, considering a biorefinery approach aiming the recovery and fractionation of aromas, to be applied as natural flavouring agents.

Regarding seafood aromas, two different approaches were developed. Due to the high organic content and diversity of sardine cooking wastewaters, a biorefinery approach was used, focused on the valorisation of its liquid effluents, not only recovering natural desirable aromas, but also lipids and proteins. Through the optimisation of an integrated coagulation-flocculation/reverse osmosis process, an efficient valorisation of sardine cooking waters (produced by the canning industry) was achieved. The implementation of this approach results in two separate products that may be used as natural additives: i) a solid fraction rich in proteins and lipids (above 20% w/w) for pet and aquaculture feed and ii) a liquid aroma concentrate to be used as a natural flavouring for food or feed products. It also prevents the production of a high-organic-load effluent that represents a high environmental burden and allows for the recovery of water for reuse in the manufacturing process. Following a valorisation approach focused on the fractionation of natural accepted aromas from off-flavours, an integrated process of organophilic pervaporation and fractionation by two-step condensation was assessed for the valorisation of aromas from liquid effluents. For this study, a model solution was successfully designed, considering the aromas present in the common raw materials, such as fish, oysters, mussels, and shrimp.

Furthermore, the support of a mathematical model for the fractionated condensation processing step enables the design of appropriate strategies for the production of aroma profiles, with different chemical families, depleted in off-flavours. This mathematical model represents a simple tool that might be used in diverse integrated processes of pervaporation–fractionated condensation, vapour permeation-fractionated condensation and also for the

stand-alone fractionated condensation step, allowing the optimisation of the process operating conditions to achieve a target composition of condensates.

Due to the lack of studies with real matrices for aroma recovery and to understand the effect of the matrix on the application of the model developed, the same integrated pervaporation and fractionation by two-step condensation was applied to sardine cooking wastewaters. Despite the high heterogeneity of the sardine cooking wastewater, the effect of the matrix did not prove detrimental, with no significant impact on the reduction of the total permeate flow rate. The mathematical model applied and validated for sardine cooking wastewaters proved to be an excellent tool to achieve the recovery of valuable aromatic condensates with a drastic reduction of off-flavour content.

To understand the applicability of the mathematical model for off-flavour removal from vapour effluents, the red wine case study was selected. The red wine effluent (headspace) has the additional complexity of presenting a co-solvent, ethanol, and huge amounts of CO_2 and off-flavours resulting from microbial activity. The fractionated condensation mathematical model was extended to two different processes: a stand-alone fractionated condensation and an integrated vapour permeation/fractionated condensation, aiming at the recovery of red wine aromas free from off-flavours. Both processes enable a good recovery of esters from the red wine model solution. However, only the vapour permeation-fractionated condensation system allows for obtaining a significant decrease in the amount of ethyl phenols.

The work developed in this PhD thesis is focused on the development of integrated membrane processing and fractionated condensation systems to produce aromatic fractions without off-flavours. These processes enable the development of aromatic profiles that can be re-included in the process or valorised as natural flavourings in the feed and food chain, depending on the complexity of the effluent to be valorised. It is also important to note that these processes enable the food and wine industries to reduce the organic load in their effluents while lowering costs and using a regenerative approach resulting in new products.

Finally, it should be emphasised that one of the major contribution of this work is the mathematical model developed to predict the fractionated condensation with minimal experimental work (total condensation experiments and determination of inert gas molar flow rate) and the use of reliable thermodynamic properties. The model has been validated for both model and real solutions and can be applied to complex effluents, including inert gases that slow down the condensation process. Additionally, due to the range of chemical families evaluated in the several studies included in this thesis, it may be concluded that this model represents a tool that can be easily applied to other industries, which might recover aromas and integrate them following a circular economy perspective.

4.2. Future Work

The present PhD thesis proposes different approaches for the valorisation of effluents from wine and food industries with different degrees of complexity. Several relevant accomplishments were obtained, opening new questions that should be addressed. To enable the application of the approaches developed at a pilot scale, the following recommendations for future work are proposed.

All integrated processes should be scaled-up and the operating conditions tested, in order to validate the model under real conditions, and address any issues that may affect productivity and cost-effectiveness, such as:

- For the coagulation/flocculation step, assess the need for adjustment of the concentration of coagulating and flocculating agents to the highest volumes of effluent;
- For the pervaporation step at pilot-scale, it is necessary to identify optimum fluid dynamics conditions in order to minimize concentration polarisation effects near the membrane surface;
- In the case study of red wine, it would be very important to validate the model developed with real matrices to validate this tool for a sector where the aroma is the key to wine quality;
- For the fractionated condensation, a proper design of the condensers is required to assure the effective condensation of high quantities of vapour, providing their adequate residence time.

Additionally, a techno-economic evaluation and a Life Cycle Assessment should be carried out for each process proposed.

Another relevant question, given the differences in the perception of aroma in the different types of consumers, will be the need to carry out sensorial analysis studies applied to the different aromatic condensates obtained, in order to understand the degree of acceptance of the products obtained, directly or incorporated in the feed or food products. Understanding the degree of preference of a panel towards flavouring would be of extreme importance for the valorisation of these condensates as by-products.

As aroma compounds are very sensitive and easily lost or degraded, the enhancement of the lifetime of these condensates is also relevant. In this work, it was evaluated the advantage of adding an antioxidant extract for the maintenance of the aromatic content during the aroma recovery process. Planning ways to increase the stability of these aroma compounds will be essential, mainly motivated by the processes of incorporation of these as flavourings in the feed or food production chains. Another solution could be by encapsulation,

usually applied for the protection and/or preservation of bioactive, volatile, and easily degradable compounds from biochemical and thermal deterioration.

An interesting technique that could be considered is the Particles from Gas Saturated Solutions (PGSS) technique, where the active compound of interest is mixed together with the coating material and CO_2 in a static mixer operating at high pressure. Then, a mixture of the active compound into the gas-saturated coating material is expanded down to atmospheric pressure through a nozzle to form solid particles. This technique has the advantage of decreasing the temperature of the encapsulation process, due to the lowering of the melting point of the encapsulating agent by the application of high pressures. Integration of sweeping gas (CO_2) pervaporation/vapour permeation process with PGSS could be a promising strategy to obtain solid particles containing the target aroma compounds with increased stability. Silica could be also used as a food-grade carrier, due to its ability to absorb moisture and to keep dry powders dispersed. Silica might be considered to produce a delivery system for aromas, increasing its physical stability.

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APPENDIX

A. Sensitivity analyses of the impact of relevant properties: saturation vapour pressure, activity coefficient and the inert gas molar flow rate

One of the difficulties experienced during this study was the need to find in the literature and databases the requested thermodynamic parameters (saturation vapour pressure and activity coefficient) of the compounds under study and to assess their reliability. Specifically, it was not possible to find values of activity coefficients at 60 °C for most aromas. Therefore, the activity coefficients of the different aromas were taken from data at 50 and 25 °C (depending on the values found in the bibliography, see in the manuscript **Table 2.6**), although recognising that this simplification introduces uncertainty. On the other hand, the inert (non-condensable) gas molar flow rate (experimentally measured) is an important parameter for the process, due to its high influence on the recovery of aromas by condensation.

A sensitivity analysis study was therefore performed in order to estimate the impact of uncertainty of these parameters on the composition of the condensates. The parameters evaluated were: 1) the saturation vapour pressure, by testing all Antoine constants reported for each aroma; 2) the inert gas flow rate, analysing the impact of a 10 % uncertainty in this parameter and; 3) the activity coefficient, evaluating the impact of a 30 % uncertainty. The percentage of variation applied to this last parameter was based on the variation found between the activity coefficient values at 25 and 50 °C, reported in the literature. Values outside the 30 % range, reported in the bibliography, were also tested, particularly in the case of 1-penten-3-ol.

Figure A.1 shows all the simulation results obtained for each aroma. To illustrate that the results of this analysis were similar for all aromas, we chose to show an aroma from each chemical family present in the model solution. The range of variation of 30 % imposed to the activity coefficient and the range of variation of 10 % of n_{inerts}, do not seem to impact substantially on the simulated results. However, when we analyse the impact of using different Antoine constants reported in the literature, we found a significant effect on the simulated values. As can be seen in **Figure A.1** a), b) and d), when different Antoine coefficients were applied in distinct simulations and compared with the experimental values, significant deviations could be observed. Briefly, this study concludes that accurate determination of saturation vapour pressure is important for consistent modelling, where the latter is essential.

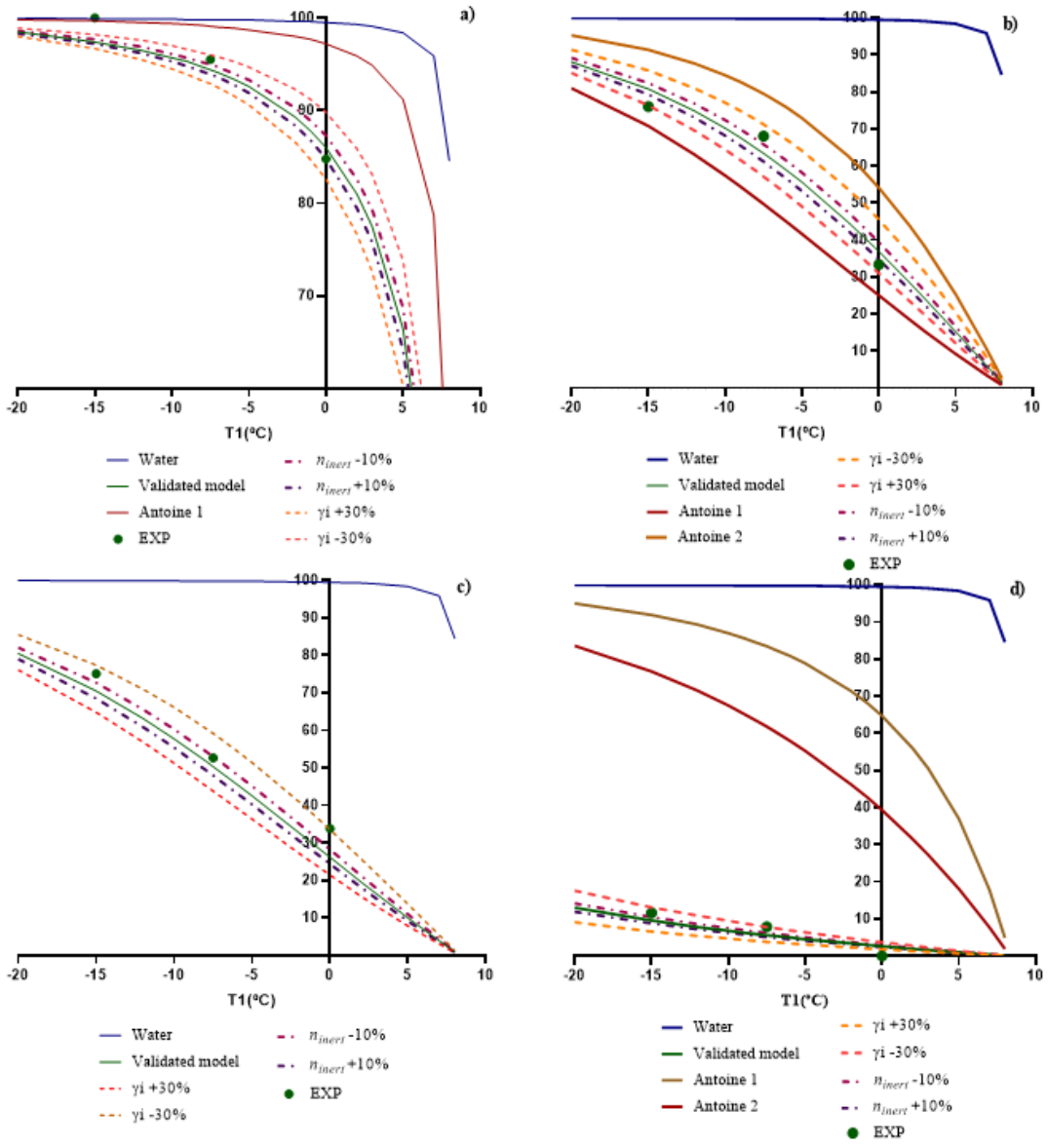

Figure A.1. Simulations obtained during the sensitivity analyses of thermodynamic parameters (saturation vapour pressure and activity coefficient) and of inert gas flow rate. Percentage of condensation of each aroma in the 1st condenser (*%condens*$_{s1}$): a) 1-penten-3-ol, b) 2-nonanone, c) heptanal and d) dimethyl disulphide. Green lines represent the validated model for each aroma (as seen in **Figure 2.16**) and blue lines represent the water condensation; other full lines correspond to simulations using other Antoine constants found in the literature for each aroma tested; dashed lines represent simulations varying the activity coefficient by 30% and pointed lines represent simulations varying the ninert gas by10 %. Dots represent the experimental data.

2022

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RECOVERY OF NATURAL FLAVOURS BY INTEGRATED MEMBRANE PROCESSING AND FRACTIONATED CONDENSATION: CASE STUDY OF SEAFOOD AND WINE INDUSTRIES